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FREEZING AND MELTING POINTS OF INSECT TISSUES[1]

BY R. W. SALT[2]

Abstract

The various tissues of two species of insects, one cold-hardy and the other not, were found to have melting points between −0.5° and −1.0° C. Because of this uniformity, the relationship between temperature and extent of ice formation, quantitatively worked out for blood of *Loxostege sticticalis* (L.) in a previous paper, may be considered representative of insect tissues in general, except possibly those of insects that withstand freezing.

Introduction

In 1926 Payne (1) reported two supercooling points in oak borers that were in winter conditon. The first supercooling point was not fatal, but the second, near −40° C., was always fatal. She states "The tissue freezing was not definitely isolated but experiments pointed to the possibility of the tissue being either fat within the cells or nervous tissue. . . . The secondary freezing point represents the freezing of some tissue other than the blood. One experiment was tried to obtain the freezing point of the nervous tissue. The central nervous systems of fifty larvae were dissected out, placed in a small tube and frozen. The undercooling point recorded was −48° C., the freezing point −45° C."

This suggestion that various insect tissues may have widely different freezing points has not received much attention, although it was shown by Salt in 1936 (3) that Payne's method of obtaining freezing points was completely erroneous. In the same paper Salt described attempts to determine the freezing points of some of the oak borers used by Payne. These were first frozen and then tested for softness at intervals as the temperature was raised. Some softening was suspected at about −11° to −13° C. in one species; another was hard at −7° C., but softened gradually above this temperature; in a third species the thorax was still hard at −2° C., but the abdomen had become soft. This melting and prodding technique was

[1]*Manuscript received September 22, 1955.*
Contribution No. 3359, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Canada.

[2]*Principal Entomologist, Field Crop Insect Section, Science Service Laboratory, Lethbridge, Alberta.*

[The December number of this Journal (Can. J. Zoology, 33: 389-448. 1955) was issued December 16, 1955.]

admittedly crude and inadequate, but . . . "appears to demonstrate a variation in freezing points of different parts of the body." Since that time the writer has had reason to doubt that the tissues of some insects at least vary much in freezing point.

The literature contains reports of studies of osmotic pressure in insect tissues, measured by the freezing point method, in which differences of the order of a degree Centigrade were found. Minor differences exist from place to place within a single organ or tissue, and from one time to another. As would be expected of excretory organs, Malpighian tubes exhibit some of the lowest freezing points and greatest variations (2). The differences are negligible, however, in terms of protection against freezing injury.

The present study of tissue freezing was long delayed by the lack of a suitable technique. When a piece of tissue is dissected from an insect the problem of handling it until the measurements are made is beset with difficulties. Chief of these is the avoidance of drying, either of the tissue itself or of the Ringer's solution if this is used. Wiping and surface drying of a small bit of tissue produces a zone of dehydration in the outer layers which rapidly affects the interior. Evaporation of Ringer's produces hypertonicity and a consequent withdrawal of water from the tissue. The freezing point is quickly depressed by any such treatment. Dissection and freezing under mineral oil has the disadvantage that the tissue fragment will almost certainly be covered with a layer of haemolymph which when frozen obscures observation of the enclosed tissue.

Although such technical problems make the precise measurement of freezing points, melting points, or osmotic pressures very difficult, the tendency towards false values can be reduced greatly by developing rapid methods of measurement. In the present studies this was readily accomplished, since it was only necessary to find whether the freezing points of insect tissues varied greatly; if they lay within a range of two or three degrees more precise measurement was unnecessary.

Methods

Specimens were dissected in Ringer's and a small bit of tissue transferred to a glass cover slip having a ring of Vaseline around the periphery. Excess Ringer's was blotted away, but a small amount was deliberately left. A second cover slip was then placed over the first, and by applying light pressure the Vaseline sealed the edge. A small amount of Ringer's solution and liquid squeezed from the tissue surrounded the flattened tissue fragment. The preparation was then placed inside and near the center of a flat cellophane dialyzing tube, about a foot long and an inch wide, with a thermojunction alongside one cover slip. One end of the dialyzing tube was closed and the other connected to a suction pump. This appressed the cellophane closely to the cover slips and ensured that no leakage would take place towards the tissue when the center portion of the tube was immersed in cold alcohol. An

alcohol bath, cold enough to supercool and freeze the tissue, consisted of a glass dish, 1.5 in. high and 5 in. in diameter, with a microscope slide fixed near the center, half an inch above the bottom. The specimen was clamped on the microscope slide and the bath placed on the stage of a dissecting microscope. Melting of the tissue as the bath warmed was observed by polarized light and at a magnification of 54 diameters. One Polaroid filter was placed at stage level above the light source and the other just below the objectives. The two filters were about 45° out of phase. Temperature was recorded continuously on a single-pen electronic recorder having a sensitivity of $\pm$ 0.15° C.

The Freezing and Melting Points

As pointed out in another paper (4), solutions have no true freezing and melting points in a strict sense. These terms as commonly used refer to the highest temperature at which freezing can occur without supercooling or at which the last crystal of ice melts. In each case the temperature is the same, and in the present problem the melting point is the more easily measured. It is important to realize that it is only the end point of a process, for melting takes place continuously with rising temperature, increasing in amount as the melting point is approached. Conversely freezing increases in amount as the temperature falls; here there is a starting point but no end point, unless it is at the eutectic temperature, a point not yet possible to measure in complex biological systems.

Results

Melting point measurements were made on a variety of tissues of non-cold-hardy *Dissosteira carolina* (L.) adults and cold-hardy overwintering larvae of *Cephus cinctus* Nort. At least three measurements were made for each tissue. The end point was not always the same over the entire area of tissue, and some judgment was necessary in recording the final temperature. However, it soon became apparent that the differences found among different tissues were of similar magnitude to the experimental error, and hence the tissues were approximately isosmotic. All had melting points between −0.5° and −1.0° C. The tissues tested were: muscle from femur, thorax, and dorsal vessel; brain; optic lobe; abdominal and thoracic ganglia; various nerve cords; fore- and hind-gut, with and without cuticular lining and muscle layers; mid-gut; peritrophic membrane; gastric caecum; salivary gland; blood; fat body; Malpighian tubes; ovary; calyx; yolk of full-grown oöcyte; accessory male sex gland; testicular tube; entire gonad (of *C. cinctus*).

The only tissues that gave any suggestion of being different were the Malpighian tubes of *D. carolina*. Some of these were completely melted at −1.5° C., but others melted between −0.5° and −1.0° C. The variation is probably to be expected because of the excretory function of the tubes, and has been noted previously in osmotic pressure studies (2).

Discussion

The two species investigated differ greatly in cold-hardiness, for *D. carolina* supercools about 5° to 8° C., *C. cinctus* about 23° to 30° C. Once frozen, however, the difference in cold-hardiness disappears. At a given temperature the extent of freezing is virtually the same in both species, and in all tissues. Neither the nervous system nor any other tissue resists freezing once ice formation has started. This is not to say that tissues do not vary in their ability to supercool. They may do so, but if so, the only one that matters is that which supercools least; when it freezes the others are inoculated and also freeze.

The relationship between temperature and ice formation has been worked out quantitatively for blood of *Loxostege sticticalis* (L.) (4) and is shown in Fig. 1. Now that it has been shown that insect tissues are nearly isosmotic the curve may be considered representative of insect tissues in general, except possibly those of insects that withstand freezing.

The amount of freezing that takes place in the body is important to an insect's survival. Many insects that are susceptible to freezing can survive it at temperatures down to about −5° C. if the exposure is very short. Usually they must be inoculated with water to induce freezing at such high temperatures. However, the ability to survive inoculated freezing drops sharply as the temperature falls. Even at −5° C. 65 to 70% of the body moisture is crystallized. Further ice formation is progressively more lethal.

In any consideration of the lethal effects of freezing at temperatures just below the freezing point the duration of exposure is very important. It is possibly true that at such temperatures the time required to reach a water–ice equilibrium is appreciable. On the other hand, the increase in harmful

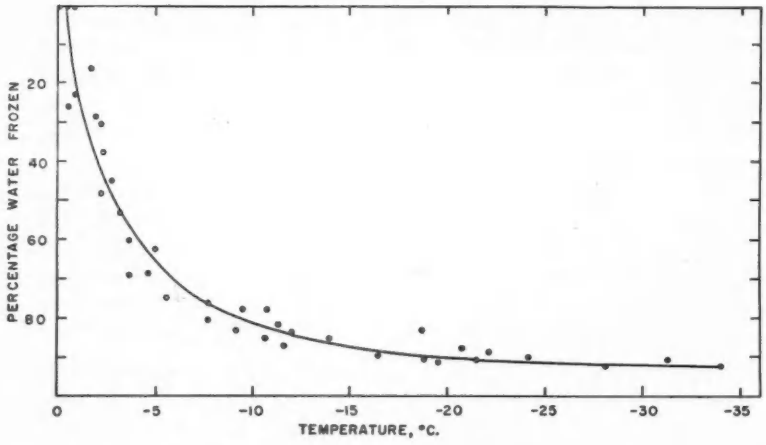

FIG. 1. Relationship between temperature and ice formation in frozen blood of *Loxostege sticticalis* larvae (based on Salt, 1955).

effects with time, which has been experimentally verified, may be caused by the time–temperature effect also known to be operating independently of freezing.

Under natural conditions supercooling takes place until the temperature falls to the supercooling point. Freezing then takes place suddenly and rapidly, with a wave of congelation sweeping through the body at a rate of a few centimeters per second. Although heat of crystallization is produced it is rapidly dissipated to the colder surroundings and in a minute or two at most the supercooling point is again reached. Thereafter the extent of ice formation is typical of the existing body temperature. In an insect that supercools as little as 10° C., 75 to 80% of the body moisture freezes. At −15°, 85 to 90% will be frozen, and at −20°, 90% or more. Since most hibernating insects supercool at least 15° C., there is no chance of their surviving freezing unless they are of the freezing-resistant type. How this small group of insects survive freezing and all others perish is not known, but it is a problem of fundamental importance that has received very little attention. If their freezing points were found to be much lower than the ones recorded here (the oak borers previously mentioned indicate this possibility), it might provide the explanation.

Acknowledgments

Criticism of the manuscript by Drs. C. W. Farstad and W. C. Broadfoot, of the Science Service Laboratory, Lethbridge, is gratefully acknowledged.

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HELMINTH PARASITES OF REPTILES, BIRDS, AND MAMMALS IN EGYPT

II. CATENOTAENIA AEGYPTICA SP. NOV. FROM MYOMORPH RODENTS, WITH ADDITIONAL NOTES ON THE GENUS[1]

By Robert W. Wolfgang[2]

Abstract

Catenotaenia aegyptica sp. nov. from myomorph rodents in Egypt is described. The genus is evaluated and *Meggittina* is regarded as a synonym. *C. oranensis* is recorded from *Psammomys obesus* from Egypt, and *C. linsdalei*, *C. peromysci*, and *C. laguri* are regarded as synonyms of *C. dendritica*.

A small, bizarre cestode was included in a collection of helminths from rodents received from Lieut.-Commander Robert E. Kuntz, U.S. Naval Medical Corps Unit 3, Cairo, Egypt. The material represents about 25 specimens which belong to the genus *Catenotaenia* Janicki, 1904. It was preserved in FAA (formalin – acetic acid – alcohol), and stained in Delafield's haematoxylin and Mayer's paracarmine. The author is grateful to Dr. Kuntz for the privilege of examining this material.

Catenotaenia aegyptica sp. nov.

Measurements of total length (1.06 to 5.60 mm.) were made from the anterior end of the scolex to the middle of the posterior end of the last segment in the strobila. The superficial appearance is roughly triangular (Figs. 5-6) with the posterior end of the worm much broader than the anterior. The lateral margins of the last proglottid are bent posteriorly, giving that end of the worm a bifurcal shape (Fig. 7). The scolex (Fig. 1) is broad without a distinct neck, has four suckers (0.12 to 0.16 mm. in diameter), and is with or without a depression, a remnant of the apical sucker. There were never more than six proglottids, and most specimens had only four, arranged as follows: an immature proglottid containing rudiments of genitalia (Fig. 1), a mature proglottid (Fig. 2) which contains testes, ovary, and yolk gland and which is broader than the immature proglottid, an intermediate proglottid (Fig. 3) with gonads and genitalia still present but with the uterine stem and branches developed, and one gravid proglottid (Fig. 7) with genitalia and testes still present but with the uterus voluminous and the shape of the proglottid altered. The gravid segment is usually partially detached from the remainder of the strobila (Fig. 6). The genital pores are regularly or irregularly alternate.

[1]*Manuscript received February 25, 1955.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College P. O., Quebec, Canada, with financial assistance from the National Research Council of Canada. This survey has been made possible by the co-operation of Dr. R. E. Kuntz, Mr. Harry Hoogstraal, and the technical assistance of Mr. G. M. Malakatis, all of the United States Naval Medical Research Unit No. 3, Cairo, Egypt.

[2]*Research Assistant and Assistant Professor of Parasitology.*

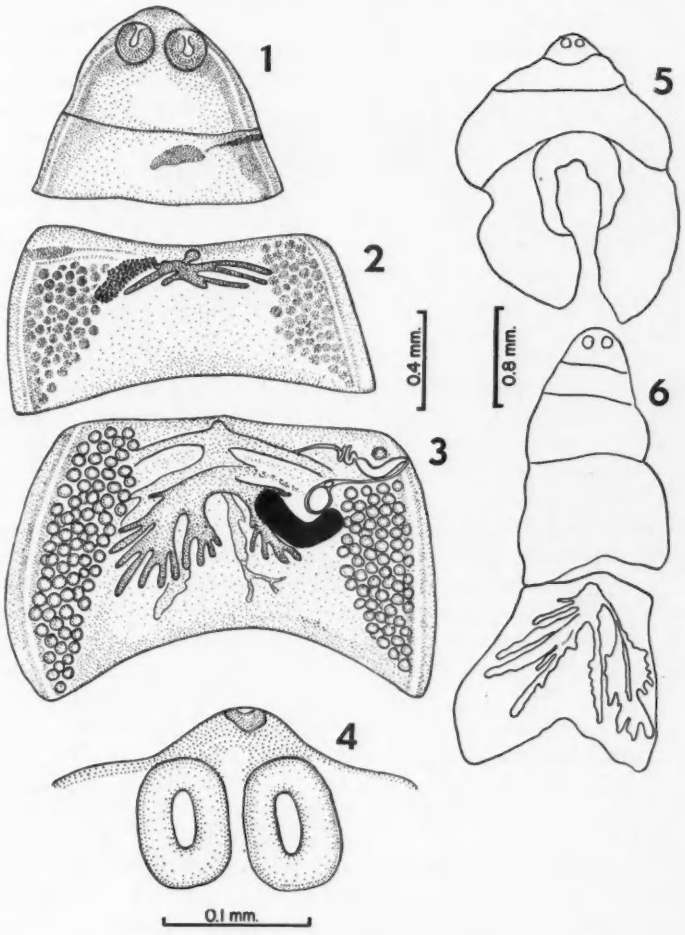
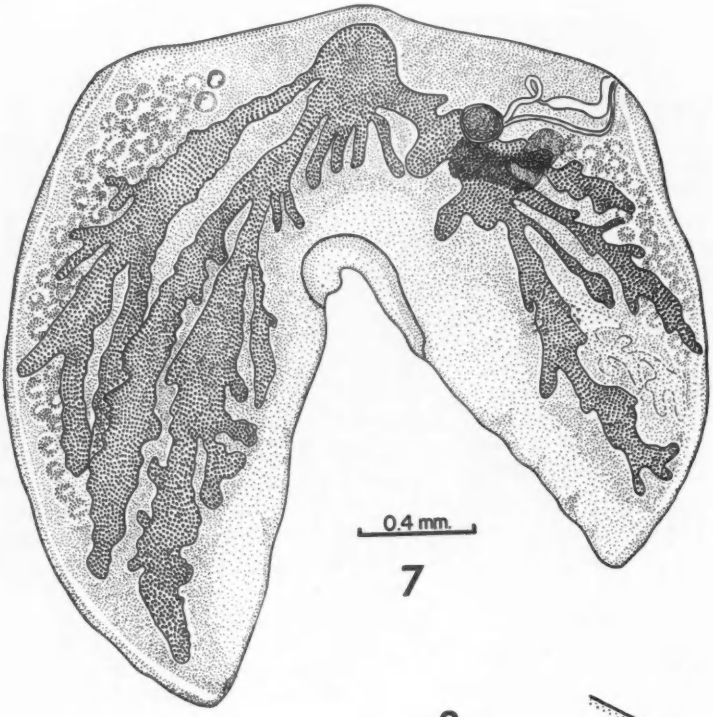


FIG. 1. *C. aegyptica* : scolex and immature segment.
FIG. 2. *C. aegyptica* : mature segment.
FIG. 3. *C. aegyptica* : mature segment.
FIG. 4. *C. baeri* : scolex.
FIGS. 5 and 6. *C. aegyptica* : variations in shape.

The genitalia (Fig. 8) are located in the anterior part of the proglottid. Both the cirrus pouch (0.18 to 0.28 mm. by 0.02 to 0.05 mm.) and the vagina open side-by-side at the genital pore. The vas deferens makes several tight turns proximal to the cirrus pouch and extends medially. The vagina lies posterior to the cirrus pouch and extends posterior to but in the same direction as the vas deferens. The vagina terminates in a large seminal receptacle, the poral part of which is much smaller than the antiporal part. It appears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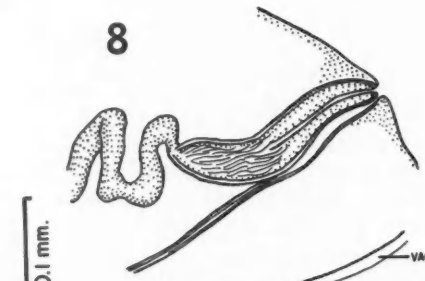

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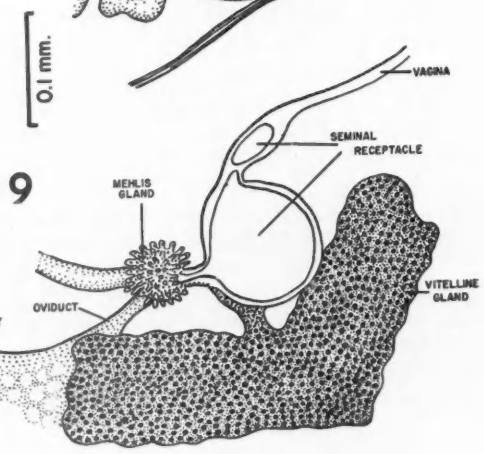
0.1 mm.
VAGINA
SEMINAL RECEPTACLE
MEHLIS GLAND
VITELLINE GLAND
OVIDUCT
OVARY

9

bipartite owing to a fold in the receptacle. In immature and mature proglottids this organ is transparent but in later segments it stains deeply as if filled with spermatozoa (Fig. 7).

The testes are in two lateral fields (53 to 108 antiporal and 40 to 89 poral), and are always compact and small in anterior segments and diffuse to transparent and large in gravid segments. The ovary is dendritic and voluminous, filling the medial space between the testes. It becomes compact and branching and is harder to see in outline in older, broader segments. The vitelline gland (Fig. 9) is large and boomerang-shaped, lying lateral to the ovary and occupying the space between the anterior-poral side of the ovary and the genitalia. In shorter segments it may be horizontally straightened rather than curved.

The uterus, as it appears first in the mature segment, has a short median stem which extends to the anterior edge of the proglottid. In the intermediate proglottid it has a short stem with four to eight lateral branches. In the gravid segment the anterior branches become voluminous and the most posterior branches are usually short. This elongation of the anterior branches modifies the shape of the proglottid causing it to bend backwards at the posterior edges to accommodate the changing shape of the branches. The shape of the gravid segment is not uniform (Figs. 5-7), and intermediate shapes between those of *C. oranensis* and *C. baeri* were observed.

The eggs of *C. aegyptica*, like those of *C. oranensis* and *C. baeri*, are not embryonated in the gravid segment. They are spherical (0.010 to 0.014 mm. diameter) with a delicate external membrane surrounding a compact granulated mass.

Hosts: *Meriones* sp., *Acomys cahirinus*, *Gerbillus gerbillus*.
Locality: Mersa Matruh, West Desert; Chamrah, Cairo, Egypt.
Location: Small intestine.
Type specimen: To be deposited in the Helminthological Collection, U.S. National Museum.

Catenotaenia aegyptica most closely resembles *C. oranensis* Joyeux and Foley, 1930 and *C. baeri* (Lynsdale, 1953). It differs from *C. oranensis* in having a much shorter uterine stem, fewer proglottids, a differently shaped vitelline gland and seminal receptacle, and is a differently shaped worm. *C. baeri* differs from *C. aegyptica* in being shorter, in having two instead of four to six proglottids, a shorter uterine stem, and fewer uterine branches (one instead of two to four); it is much different in shape, and has a differently shaped vitelline gland and seminal receptacle. All three species are similar in the anatomy of the mature proglottid; e.g., the arrangement of the testes in lateral fields, a medially placed dendritic ovary, and cirrus, vagina, uterus, and scolex which are similar in all species. These three species are closely related morphologically and all come from rodent hosts in Africa.

FIG. 7. *C. aegyptica* : gravid proglottid.
FIG. 8. *C. aegyptica* : terminal genitalia.
FIG. 9 *C. aegyptica* : female organs.

This genus has been placed in several families (Taeniidae, Dilepididae, and Anoplocephalidae) prior to 1950. Spasski (18) placed the genus in a separate family Catenotaeniidae, subfamily Catenotaeniinae Spasski, 1949. Wardle and MacLeod (20) independently created the same family for the genus, apparently being unaware of Spasski's family as they neither mention it in their discussion nor include a reference to his paper (17) in their bibliography. Lynsdale (9) criticized the erection of the family and pointed out that the reasons given by Wardle and MacLeod for creating it parallel those given by Joyeux and Baer (6) for including it in the family Anoplocephalidae. The reasons given by Spasski for erection of the family Catenotaeniidae are similar to those presented by Wardle and MacLeod. The author agrees with Lynsdale that there is at present no substantial proof supporting the separation of the genus from the Anoplocephalidae, and that its separation serves no useful purpose. He does feel, however, that there is some merit in retaining the subfamily Catenotaeniinae Spasski, 1940. Although the genus is similar in most respects to other genera of the family Anoplocephalidae, it differs in the basic characteristics reported by Joyeux and Baer (6) in describing the life cycle of *C. pusilla* (Goeze, 1782).

Akhumyan (1) (according to Dollfus (4)) has split the genus *Catenotaenia* into two separate genera, *Catenotaenia* and *Skrjabinotaenia*. The latter genus was created for *C. oranensis* and *C. lobata* primarily on the basis of the testes being distributed in two fields lateral to the ovary. This does not seem to be a reliable character when one considers the similarity of *C. lobata* to other species with posterior testes and the fact that Baer's (2) description showed that the testes unite posterior to the ovary, as they do in many other species. The genus *Skrjabinotaenia* as described should be considered a synonym of *Catenotaenia*.

From its description, *Meggittina baeri* Lynsdale, 1953, from "house and granary rats" in Rhodesia would seem to be a species of *Catenotaenia*. The author has had the privilege of examining the type specimen (from the London School of Hygiene and Tropical Medicine), and disagrees with Lynsdale's description in several respects.

She describes, quite accurately, the form of this worm as a distortion of the typical cestode proglottid which has become so broadened that lateral characters lie anterior in the gravid proglottid. In her comparative table, however, her reference to testes anterior to the ovary is misleading. The anterior portion of the worm or any part of it must be relative to the orientation of the worm as a whole, that is, the portion lying proximal to the scolicial end, be it gravid, immature, or mature proglottid, must be the anterior edge and the portion lying along a free margin the lateral edge. The testes lie along the free border of the proglottid, not the attached border and must, therefore, be lateral. Her reference to a bipartite seminal receptacle (receptaculum seminis) as being distinctive of this genus is incorrect as shown by an examination of specimens of *C. oranensis* Joyeux and Foley, 1930, which has similar characteristics (Fig. 14).

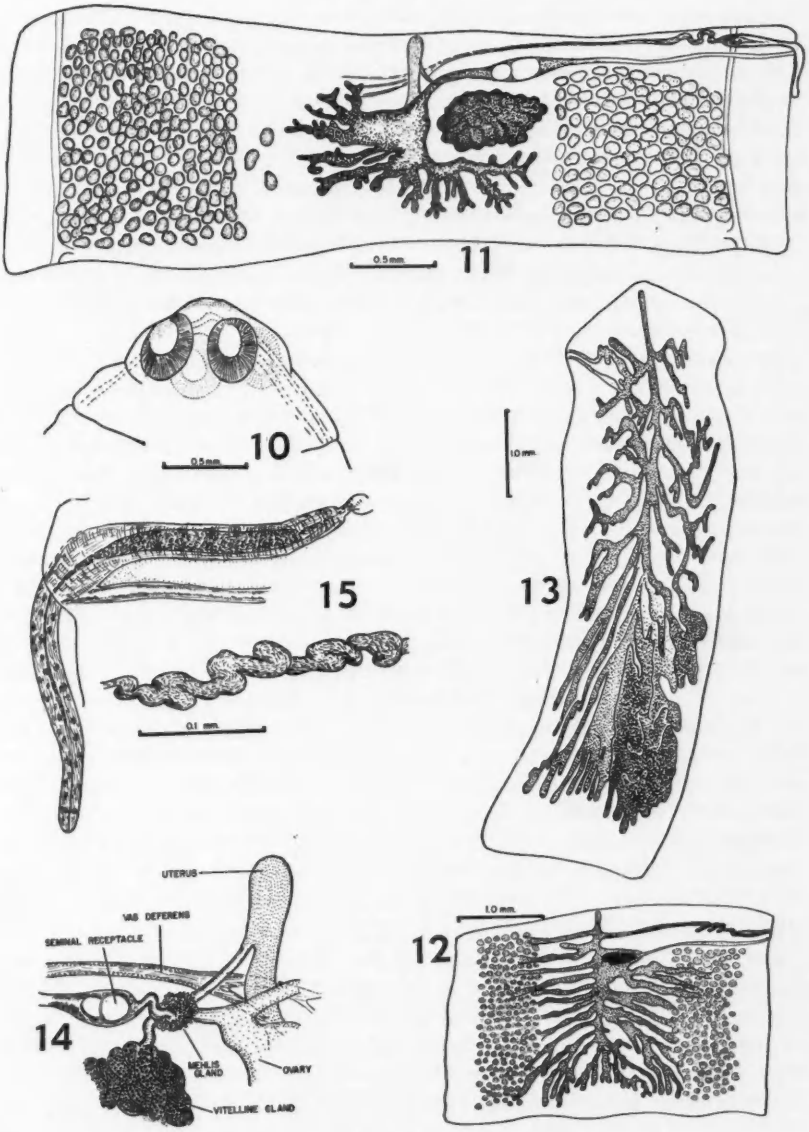

FIG. 10. *C. oranensis* Joyeux and Foley, 1930 : scolex.
FIG. 11. *C. oranensis* : mature proglottid.
FIG. 12. *C. oranensis* : early development of the uterus.
FIG. 13. *C. oranensis* : gravid proglottid.
FIG. 14. *C. oranensis* : female organs.
FIG. 15. *C. oranensis* : cirrus pouch, vagina, and vas deferens.

Lynsdale remarks on the similarity of *C. oranensis* to *M. baeri* but considers the differences — which the author considers superficial — to be sufficient to separate it from the genus *Catenotaenia*. In the type specimen of *M. baeri* the author has found a distinct but poorly developed apical sucker (Fig. 4), which is characteristic of some species of *Catenotaenia*. The new material from Egyptian rodents constitutes a species intermediate both in anatomy and superficial shape and indicates clearly the relationship of Lynsdale's species with others of the genus *Catenotaenia*. The author, therefore, suggests a new combination: *Catenotaenia baeri* (Lynsdale, 1953).

In Dr. Kuntz' collection there were numerous specimens of *C. oranensis*, all from *Psammomys obesus* in Egypt. These specimens represent a new host and distribution record for this species. Some of the specimens stained beautifully with Grenacher's borax carmine and the anatomy of the worm could be studied in detail (Figs. 10-15). Joyeux, Baer, and Gaud (7) give the genital pores as irregularly alternate. In nearly all specimens studied by the author, however, they were regularly alternate. Joyeux and Foley (8), in the original description, describe considerably smaller specimens than those reported by Joyeux, Baer, and Gaud (7); the number of testes and the size of the cirrus pouch varied considerably between the two descriptions. The material from *Psammomys* resembled most closely that of Joyeux, Baer, and Gaud (7). In some specimens there was a sucker-shaped depression at the apical end of the scolex; there seemed to be a concentration of nuclei about the depression although no obvious muscle fibers were associated with it. In *C. oranensis*, as in the species described here, the seminal receptacle is transparent in the immature and mature proglottids, becoming opaque and deeply staining in segments where the uterine branches begin to appear. The shape of the gravid uterus in *C. oranensis* is very irregular. Posterior branches are long and tend to change the shape of the proglottid from square to longer than wide. There seems to be a change in shape of the uterus as it develops; the eggs appear to move from the main stem into the posterior lateral branches. In these specimens, as in those described by other workers (8, 7), no eggs were fully embryonated. The most gravid segments begin breaking away from the strobila before embryonation is complete.

Recently, Smith (16) and Schad (15) have suggested, simultaneously, that *C. linsdalei* MacIntosh, 1941, is a synonym of *C. dendritica* (Goeze, 1782). Smith describes two new species, *C. peromysci* and *C. laguri* from Wyoming rodents. He recovered what he describes as immature *C. dendritica* from the same host that harbored *C. laguri*, but did not compare the latter with *C. dendritica*. His reference to eggs "0.060 to 0.062 mm." is not supported by their size in his figure (16, p. 345). The reference to "vagina and vas deferens not parallel" in distinguishing it from other species is not supported by a comparison with MacIntosh's (10) figure of *C. linsdalei*. From a review of the data presented in Smith's paper the author can find no differences separating the two species and concludes that *C. laguri* is a synonym of *C. dendritica*. *C. peromysci* differs from *C. laguri* only in having testes divided

into two fields behind the ovary which are joined posteriorly in the proglottid. In the author's opinion this is a doubtful character depending primarily on the development of the uterine stem in a particular proglottid, and in the absence of other differentiating characters it would seem that this species is also a synonym of *C. dendritica*. The host distribution and the geographical range of *C. dendritica*, as reported by other workers, further suggests that Smith was working with this species.

A variety of characters has been used to separate the species of *Catenotaenia*: strobilar length, spacial arrangement of organs in both gravid and mature segments, terminal genitalia, excretory system, host and geographical distribution, and egg morphology. The author proposes to discuss these criteria on the basis of examination of *C. oranensis*, *C. aegyptica*, *C. baeri*, and *C. dendritica*, and in the light of existing literature.

Neither strobilar length nor strobilar shape is a good criterion for supporting species relationships unless the latter character is sufficiently constant to permit unquestioned recognition. Clark (3) has recently described *Eurycestus avoceti* Clark, 1954 from the avocet (Aves). This is remarkably similar to *C. baeri* in shape but differs distinctly in morphology. A variety of shapes is represented in the genus *Catenotaenia*: *C. dendritica* and species most closely allied to it have long strobilae in which both mature and gravid proglottids are longer than wide. *C. oranensis*, *C. aegyptica*, and *C. baeri* have relatively shorter strobilae and are wide forms in which the gravid segment becomes radically changed in form from that of the mature proglottid. Spasski (19) has reported that there is a different metameric growth rate in short and long tapeworms, the shorter ones growing more slowly than the longer.

The most confusing character on which to base taxonomic conclusions is, perhaps, the spacial arrangement of organs. Many of the differences between *C. dendritica* and *C. linsdalei* are resolved when differences in the state of contraction of specimens are taken into account. A contracted segment of these species differs distinctly in appearance from a relaxed one. Organs which appear widely separated and relatively small in extended proglottids appear crowded and much larger in contracted ones.

One character upon which many authors have relied is the position of the testes. Testes have been described as arranged in two basic patterns: (1) behind the ovary, divided into two lateral groups which unite posterior to the uterine stem or in a single undivided posterior group, and (2) in two distinct lateral fields extending from the anterior edge of the proglottid to the posterior edge on either side of the ovary. In *C. lobata* Baer, 1925 the testes have the latter arrangement but Baer (2) shows them as united posterior to the ovary.

In his original description of *C. geosciuri* Ortlepp, 1938, Ortlepp (11) shows the testes divided into two fields behind the ovary. Joyeux and Baer (6) show them in a single field with a trace of the uterine stem appearing in their midst. In species where they occur behind the ovary the arrangement of the testes is irregular. Figures of some specimens of *C. dendritica* and *C. linsdalei* (synonym of *C. dendritica*) show them sometimes with scattered testes lying

intermediate between the two fields. Smith's (16) *C. laguri* and *C. peromysci* are identical except that in the former the testes lie in one field behind the ovary and are divided in the other. In *C. pusilla* (Goeze, 1782), which has been studied thoroughly, the testes have been figured only in an undivided field.

The author is of the opinion that too much emphasis has been placed on the character of testes divided into two fields or united in one when they occur behind the ovary. The testes in these species lie dorsal to the uterus or on either side of it. In all species of *Catenotaenia*, as the uterus grows the testes are pushed toward the lateral excretory ducts. The irregular distribution of testes in species where they have been described as occurring in two groups posterior to the ovary suggests that the size of the uterine stem and the development of the uterus determines their position. Another, and probably as important a factor in disposition, is the pressure applied to a strobila in fixing or mounting under which the testes may be displaced to either side of the central uterine stem.

The number and shape of testes are variable, as demonstrated by Dollfus's (4) table of mensural and meristic characters. Rausch (13) and Rausch and Schiller (14) have shown this character to be unreliable for other species of cestodes.

The morphology of the terminal genitalia is sufficiently characteristic for each species to warrant its use in taxonomy. The position of the genital pore (whether at the anterior end or in the anterior half of the proglottid) separates species of *Catenotaenia* into two natural groups, based on other characters. The cirrus pouch and its morphology — but not its size — is a useful character. In these species there are two types of vagina, thin-walled and thick-walled. The thin-walled vagina appears to be made up of cuticle continuous with that covering the strobila. The thick-walled vagina is constructed of densely staining cells which surround the lumen.

The excretory system for all species except *C. lobata* has been described as having simple, longitudinal canals. In *C. lobata* Baer (2) refers to a network of canals with secondary anastomosing. Dowell (5) described *C. californica* Dowell, 1953 as lacking a dorsal, longitudinal excretory canal, which is a useful character in separating this species from *C. dendritica*.

Host distribution and host specificity are insufficiently defined to use as criteria in separating species. *C. dendritica* is recorded from a large number of myomorph and sciuromorph hosts throughout the northern hemisphere. Species with long and thick-walled vaginas have been reported exclusively from sciuromorph rodents; *C. chabaudi* Dollfus, 1953 and *C. geosciuri* have been reported from similar hosts (*Xerus* species) in Africa. In Dr. Kuntz' collection of worms from Egypt only *C. oranensis* and *C. aegyptica* were recovered although a larger number of species of *Catenotaenia* is reported from Africa. *C. oranensis* has been reported only from *Meriones* in Algeria although it has occurred only in *Psammomys* in our collection. *Meriones* contained *C. aegyptica*. Thus, only the morphology, and not the host specificity,

appears to be reliable in determining the identity of species of this genus. It is interesting to note, however, that the majority of species occur in Africa. None has been recorded from South America and Australia, and none has been recorded from hystricomorph rodents.

The size and form of the egg have been described for some species. Where these are known they are useful in determining species.

In one species, *C. chabaudi*, the presence of a well-defined apical sucker on the scolex is diagnostic. The apical sucker is a larval character in this genus. Joyeux and Baer (6) have described it for the larva of *C. pusilla* in which it disappears as the worm matures. The author has seen vestiges of this organ in *C. oranensis*, *C. aegyptica*, and a well-defined sucker in *C. baeri* (Fig. 4). In the first two species it was not found in all specimens. It is doubtful whether a retained larval character in the adult should be too greatly relied upon for diagnostic purposes. In *C. chabaudi*, however, it appears to be constant.

The characteristic branched uterus is peculiar to this genus within the Anoplocephalidae. It is Taenia-like in that it has a central stem with lateral branches. For that reason taxonomic procedure has been influenced by that of the genus *Taenia*. The uterus appears first in the mature proglottid and develops rapidly thereafter. The lateral branches may be simple as in *C. dendritica* to highly dendritic as in *C. pusilla*. In any species it is difficult to predict the pattern of branching although the number of branches appears to be relatively constant for any given species. In discussing the taxonomy of the genus Rausch (12) states:

> "It appears that the gravid segments in some cases show specific features more readily recognized than those seen in mature segments alone . . ."

The author is in agreement with Rausch. The gravid segment is not subject to the same distortion as other proglottids in the strobila and thus presents a reasonably stable basis on which to identify species. Fig. 16 is a comparison of typical uteri represented by several species of *Catenotaenia*. All drawings are diagrammatic and none is to scale. Figures for *C. geosciuri*, *C. dendritica*, *C. pusilla*, and *C. lobata* are from Joyeux and Baer (6).

C. dendritica and *C. geosciuri* have similar uterine development but differ on the basis of vaginal morphology. *C. pusilla*, like the previous species, has a long uterine stem, the lateral branches of which may become extremely dendritic. It has fewer branches than either *C. dendritica* or *C. geosciuri*. *C. lobata* has a shorter uterine stem than *C. pusilla* and a similar number of lateral branches. The anterior end of the uterus forms a definite pocket; the posterior branches are more complex and longer than those of *C. pusilla*. As in the following species but unlike the previous one, its genital pore is located at the anterior end of the lateral margin of the proglottid. In *C. oranensis* the uterine stem is still shorter. The anterior lateral branches remain short and the posterior branches become greatly elongate so that the gravid segment becomes much longer than wide. It has fewer branches than *C. lobata*. *C. aegyptica*, like *C. oranensis*, has an anterior pocket on the uterine stem.

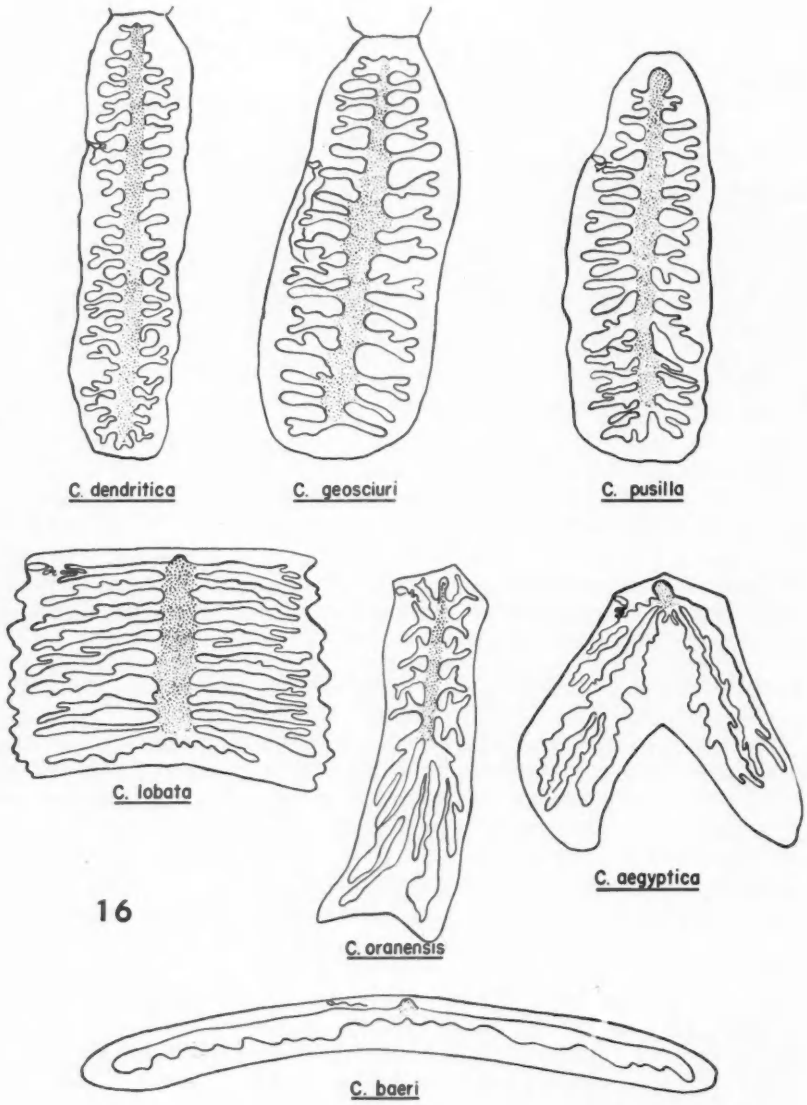

FIG. 16. Comparative development of the uterus in some representative species of the genus.

The anterior branches, however, become greatly elongate and the posterior branches remain short so that the gravid segment becomes forked in appearance. It has fewer lateral branches than *C. oranensis*. In *C. baeri* the stem is reduced to the anterior pocket and the uterine branches are limited to one very long lateral branch on either side of the pocket.

In *C. dendritica* through *C. lobata*, the lateral branches are finely dendritic distally. In *C. oranensis* the anterior branches remain finely dendritic but the posterior branches become pocketed or coarsely dendritic and the shape of these long branches is very irregular. In *C. aegyptica* only the most posterior branches remain simple in structure where they are present. In *C. baeri*, with only a single, lateral branch on either side of the stem, the branches are like the voluminous branches of *C. oranensis* and *C. aegyptica*.

By their morphology and their distribution, all of these species are clearly related. A future knowledge of their life cycles may determine how closely related they are. The development of the uterus seems to indicate that *C. lobata* is an intermediate species between those with long uterine stems and with testes confined behind the ovary and those with shorter uterine stems and testes arranged in two distinct lateral fields.

The characters which appear to the author to be of greatest taxonomic value are: the morphology of the uterus and genitalia, but not their size; the excretory system and the shape of the worm when considered with other constant characters. The testes, whether in two distinct lateral fields or lying in one field behind the ovary, will separate the two major groups of species of *Catenotaenia*. An attempt to show more clearly how these species are related is made in Table I.

C. chabaudi Dollfus, 1953 is described as having an apical sucker. It also possesses a somewhat different uterine formula than that given for *C. geosciuri* which it most closely resembles. Both species come from similar hosts. Dollfus believes that Joyeux and Baer (6) may have been working partially with *C. chabaudi* in their specimens of *C. geosciuri*; this would account for the wide variation in their figures of this species (12–18 by Joyeux and Baer (6) as compared with 33–40 by Ortlepp (11)).

C. californica Dowell, 1953 is related closely to both *C. linsdalei* (adend.) and *C. pusilla;* it more closely resembles the former. It is difficult to determine from Dowell's (5) figures how many branches there are in the type specimen. The size of the ovary suggests that it is voluminous although not more so than some figures show in *C. dendritica*. The character of the uterus developing in the mature proglottid is shown by Yamaguti (22) for *C. ris* (synonym of *C. dendritica*) as developing in front of the ovary. The use by Dowell (5) of the spatial arrangement of the female organs and of the testes as distinguishing characters is unconvincing. Dowell's reference to this species as having no dorsal excretory canal is, however, reliable as this character is constant in all of his material.

Both *C. rhombombydis* Schultz and Landa, 1934 and *C. cricetorum* Kirschenblatt, 1949 are described from rodents in Europe. The description

TABLE I

SPECIES OF *Catenotaenia*

Species	No. of uterine branches	Vagina	Gravid segment
Genital pore in anterior half of segment, testes behind ovary, uterine stem long			
C. dendritca (Goeze, 1782)	30-45 × 2	Thin-walled	Longer than wide
C. pusilla (Goeze, 1782)	9-13 × 2	" "	" " "
C. californica Dowell, 1952	25-30 × 2	" "	" " "
C. rhombombydis Schulz & Landa, 1934	22-28 × 2		
C. cricetorum Kirschenblatt, 1949	20-30*		
C. geosciuri Ortlepp, 1938	33-40 × 2**	Heavy-walled	" " "
C. chabaudi Dollfus, 1953	19-20 × 2	" "	" " "
C. reggiae Rausch, 1951	15-20 × 2	" "	" " "
Genital poreat anterior end of lateral margin, testes in two distinct fields, one on either side of the ovary but may be united posterior to it; uterine stem shorter than in previous group			
C. lobata Baer, 1925	12-20 × 2	Thin-walled, short	Square to slightly longer than broad
C. oranensis Joyeux & Foley, 1930	9-10 × 2	Thin-walled, short. Posterior branches long	Longer than broad
C. aegyptica sp. nov.	2- 4 × 2	Thin-walled short. Anterior branches long	Bifurcal
C. baeri (Lynsdale, 1953)	1 × 2	Thin-walled short	Much wider than long

** Dollfus (4) lists C. cricetorum as having 20 to 24 branches by one author and 35 to 38 by another. He does not indicate whether this number represents the number of branches on either side of the uterine stem or only on one side. The two ranges of uterine branches and the general morphology in Dollfus' table suggest that two species may be involved here. The lesser range is very close to C. rhombombydis and the greater to C. dendritica.*

*** In a footnote, Dollfus (4) points out the discrepancy between the number of uterine branches described for C. geosciuri by Joyeux and Baer (6) and those described for the same species by Ortlepp (11). The author has given Ortlepp's figures in the table. Those given by Joyeux and Baer are closer to the number cited by Dollfus for C. chabaudi.*

of neither is available to the present author. Dollfus' table shows, however, that these species may be closely related to *C. pusilla* or *C. dendritica* because both their measurements and distribution coincide with these species.

Rajotaenia gerbilli Wertheim, 1954 is similar, superficially, to *C. aegyptica* although Wertheim's (21) description indicates several important differences, one of which in particular (the lack of a cirrus pouch) could exclude *Rajotaenia* from close association with *Catenotaenia*. Wertheim makes no mention of any relationship which her species may have with this genus. *R. gerbilli* is described (21, p. 446) as having a neck, although the figure (21, p. 447) shows no such structure. She describes her species as having regularly alternating genital pores although in *C. aegyptica* the pores may be either regular or irregular depending upon the specimen studied. In Wertheim's description of the uterus, which otherwise agrees essentially with that of *C. aegyptica*, she states (21, p. 448):

"Each lobe consists of three transverse, very irregular sacs interconnected by some of the numerous sac-like expansions arising from their sides."

The uterus in *C. aegyptica* develops in typical *Catenotaenia* style, from several branches originating along the short median stem. Each branch becomes filled with eggs and the pattern of the definitive uterus is variable. Each branch has secondary branches which tend to overlap with the branches contiguous to them, and give the appearance of anastomosing, although actually each branch is distinct from the others. This dendritic condition is repeated in *C. baeri* and *C. oranensis*. The arrangement described by Wertheim would be somewhat like the tubular network typical of some Anoplocephalines, e.g. *Andrya* and *Moneocestus*. Apparently, Wertheim's specimens were always four-segmented, whereas in *C. aegyptica*, four, five, and six segments were observed; the last proglottid was always bifurcal like that of *R. gerbilli*. The cirrus pouch, which is lacking in Wertheim's specimens, is distinct in *C. aegyptica* and becomes larger as the segment becomes gravid (she shows a similar change in the size of the cirrus in her figure). Her description of the disappearance of the germovitellarium (vitelline gland) and the reduction in the number of the testes differs from what takes place in *C. aegyptica*. In the latter species the vitelline gland remains present although it may be partially hidden by the gravid uterus; the testes also are present in the gravid segment, although they are nearly transparent, much larger in size, and pushed laterally, contiguous with the lateral excretory vessels. In *C. aegyptica* the excretory vessels are simple lateral canals as in *C. oranensis* and *C. baeri*, whereas in *R. gerbilli* there are "twenty longitudinal, anastomosing vessels distributed over the whole width of the worm."

The egg of *R. gerbilli* differs from that of *C. aegyptica* in that no embryo such as is described by Wertheim could be seen in the latter species.

Both worms come from similar hosts (*Gerbillus pyramidum* for *R. gerbilli* and *Gerbillus gerbillus*, *Acomys cahirinus* and *Meriones* sp. for *C. aegyptica*), and from contiguous localities.

Generic Diagnosis

Family Anoplocephalidae, subfamily Catenotaeniinae (with uterus without a tubular network, uterine capsules, or paruterine organ, but formed of branches).

Scolex unarmed, with or without an apical sucker. Genital pores irregularly alternate lying in the anterior half of the proglottid lateral margin. Length of the strobila is variable; shape of proglottids from longer than wide to wider than long. Testes numerous and arranged either lateral to the ovary or posterior to the ovary in one or two fields. Ovary dendritic, yolk gland dendritic or compact but of definite shape, lying at the poral side of the ovary. Genitalia open together at the genital pore. Cirrus sac either straight or curved and vas deferens straight throughout most of its length, but may be coiled proximal to cirrus pouch. Vagina thin or thick-walled, long or short. Seminal receptacle large and may appear either uni- or bipartite. Uterus with median stem of variable length and shape with uterine branches of

characteristic number for each species. Eggs may or may not be embryonated. Excretory vessels anastomosing or simple canals.

Distribution: Africa, Eurasia, and North America.

Hosts and location: Intestine of myomorph and sciuromorph hosts.

Type species: *C. pusilla* (Goeze, 1782).

Life cycle known for *C. pusilla* only. This species has a "mesocercous" larva developing in Tyroglyphid mites (6).

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DOCHMOIDES YUKONENSIS SP. NOV. FROM THE BROWN BEAR (URSUS AMERICANUS) IN THE YUKON[1]

By Robert W. Wolfgang[2]

Abstract

Dochmoides yukonensis sp. nov. from the brown bear, *Ursus americanus*, is described, and the status of the genus discussed.

During the summer of 1954 the intestines of two brown bears (*Ursus americanus*) from the Yukon were examined for helminth parasites. The intestines of one bear contained no helminths and those of the second only seven hookworms. These worms belong to the genus *Dochmoides* Cameron, 1924 and represent a new species for which the name *D. yukonensis* is proposed.

The name *Dochmoides* was selected by Cameron (3) for *Uncinaria stenocephala* Railliet because the type of the genus *Uncinaria* Froelich is unrecognizable. Looss (6) had reviewed the status of the genus prior to establishing the validity of *Agchylostoma duodenale* Dubini which had previously been an *Uncinaria*. Although he redescribed the genus *Uncinaria* from Froelich's and Goeze's descriptions and figures of *Ascaris criniformis* he indicated that his diagnosis was provisional. He arrived at his decision through a complicated system of reasoning and stated three times in his discussion that the genus *Uncinaria* cannot be recognized on objective grounds, the only satisfactory basis for recognizing a genus.

According to Froelich (*in* Looss (6)) the genus *Uncinaria* is based on *U. vulpis* from foxes in Europe. Froelich also included *Ascaris criniformis* of Goeze as a second species, which was described from badgers (*Meles taxes*). Looss was of the opinion that Froelich's description and diagnosis of the genus was based on *Ascaris criniformis*. However, as the type specimen of *A. criniformis* has been lost, as well as that of *U. vulpis*, it is impossible now to make an accurate diagnosis of either. Rudolphi described *Strongylus criniformis* from the badger, the type host of *Ascaris criniformis*, and Railliet, upon re-examining Rudolphi's specimens, found them to be conspecific with his own *U. stenocephala*. According to Looss (6) Railliet proposed that this species become a synonym of *U. criniformis*. Although Looss accepted Railliet's diagnosis he was not convinced that what Rudolphi described as *S. criniformis* was identical with *A. criniformis* of Goeze. *U. stenocephala* was described in such detail that Looss was able to redescribe the genus *Uncinaria* on the basis of *U. criniformis* = *stenocephala* Railliet. This is the present basis for recognizing the genus *Uncinaria*.

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Looss thus satisfied the requirements of his immediate purpose. He separated *Uncinaria* from *Agchylostoma* and concluded by stating that if his classification were not satisfactory he could see no basis on which it could be retained on objective grounds.

Cameron (3) was unable to recognize the genus *Uncinaria* as described by Froelich and chose to select the first recognizable species assigned to this genus, *U. stenocephala* Railliet, as the type for a new genus, *Dochmoides*. Unfortunately, *Dochmoides* has not become accepted in the literature.

Ransom (7) maintained that *U. criniformis* could be separated from *U. stenocephala* although Cameron (3) could find no morphological difference between the species in badgers and those in foxes; Looss and Railliet both considered the two species conspecific. In reviewing the controversy of the validity of *U. criniformis* versus *U. stenocephala* Baylis (1) agrees with Cameron (3) that Looss was uncertain that Rudolphi's *Strongylus criniformis* and *Ascaris criniformis* of Goeze are identical. Baylis is critical of Ransom's defence of *U. criniformis* as being distinct from *U. stenocephala* because he is of the opinion, as is the present author, that Ransom used largely subjective characters as the basis of his opinion. Fülleborn (5) refers to the conspecificity of *U. stenocephala* with *U. polaris* and resolves the differences which have been described in separating these species on the basis that the dorsal view of the buccal capsule figures given by various authors be ignored. His reason for this is that the pressure of the cover glass and the angle made by the capsule is so variable that this view is unreliable for diagnostic purposes. Baylis (1) after examining a large series of specimens from foxes, wolves, dogs, and badgers concluded that he could see no difference between worms from different hosts, although he did not consider *U. criniformis* to be the valid name. Although he criticized the basis on which Looss considered *Uncinaria* to be valid he did not accept *Dochmoides* as the generic name.

The author has had the privilege of examining several collections of *Uncinaria* species from *Vulpes lagurus*, *Canis vulgaris*, and *Meles taxes*. The first two collections are listed under the name of *U. stenocephala*. The specimens from *Meles*, consisting of two males and two females collected in France, are listed as *U. criniformis*. There are no recognizable differences which would separate them and the author concludes that they are conspecific. This is in agreement with Cameron (3), Looss (6), Railliet (*in* 6), and Baylis (1). The specimens were made available to the Institute of Parasitology by the Curator of the Helminthological Collection of the United States Museum of Natural History.

Recently, Biocca and Bronzini (2) have revived the controversy of the status of *U. criniformis* and *U. stenocephala*. They maintain that the two species can be separated on the basis of the bursa characters even although others, working independently, have been unable to do so. Biocca and Bronzini state further that, as *Uncinaria* is closely related to *Ancylostoma*, it is probably very host specific; that Looss considered *U. criniformis* valid; and that care should be exercised in admitting species into synonomy, especially

when they are not described in great detail. The writer finds it difficult to justify the reasoning of these two authors, and has failed to find any literature indicating any great host specificity among species of *Ancylostoma*. While Looss did accept the validity of *U. criniformis*, he agreed with Railliet that it was conspecific with the latter's *U. stenocephala*. The writer is of the opinion that unless species can be recognized on Looss' *objective* grounds, they are unacceptable.

On the basis of the above discussion the author feels that Cameron's designation of *Dochmoides* is valid. *U. vulpis*, the type of the genus *Uncinaria*, is not recognizable. *U. criniformis* (*Ascaris criniformis* of Goeze) is no more to be recognized than *U. vulpis* because its type specimens have been lost also. Baylis (1) agrees with this view. Looss' recognition of *U. criniformis* is based on Railliet's conclusion that *U. stenocephala* is conspecific with *Strongylus criniformis* of Rudolphi. The first well-described species is *U. stenocephala* Railliet which Looss used to redescribe the genus *Uncinaria*.

Regardless of the nomenclature, *Uncinaria* Looss (not Froelich) is based on the type *U. stenocephala* Railliet, not on Froelich's *U. vulpis*. According to the Rules of Nomenclature this is an irregular arrangement. Cameron's *Dochmoides* is identical in arrangement and fulfills the requirements for establishing generic names. The present author, therefore, must accept Cameron's name.

Dochmoides yukonensis sp. nov.

The description is based on seven worms, two males (10.50 to 12.94 mm. in length by 0.52 mm. in width), and five females (14.25 to 17.12 mm. in length by 0.49 to 0.60 mm. in width). The cuticle is finely transversely striated throughout the length of the worm.

In these specimens the head is bent dorsally and the angle is not uniform. The buccal capsule (Figs. 1–4) is similar structurally to that figured by other authors for *D. stenocephala* and is larger in females (0.23 to 0.28 mm. long and 0.15 to 0.19 mm. wide at the mouth and 0.08 mm. wide at the base).

The oesophagus is approximately the same length in both sexes (0.86 to 1.15 mm. long) and is typical of the shape of this organ in other species. It is constricted at the level of the nerve ring (0.64 to 0.75 mm. from the anterior end of the worm). The excretory pore lies slightly anterior to slightly posterior to the nerve ring. Several valves represent the sphincter at the posterior end of the oesophagus. Cephalic glands are of the *Ancylostoma* type.

The tail of the male (Fig. 2) consists of a small genital cone enclosed by the lobes of the voluminous bursa. Prebursal papillae are similar to those described for *D. stenocephala*. The bursal formula is similar to that described for other species of the genus. The dorsal ray (Fig. 2) is bifid during its posterior fourth with each fork tridigitate. The two lateral lobes are symmetrical. The spicules (Fig. 3) are long (1.65 to 1.75 mm. long), filiform and heavily sclerotized. The gubernaculum is broadest posteriorly (0.20 mm. in length).

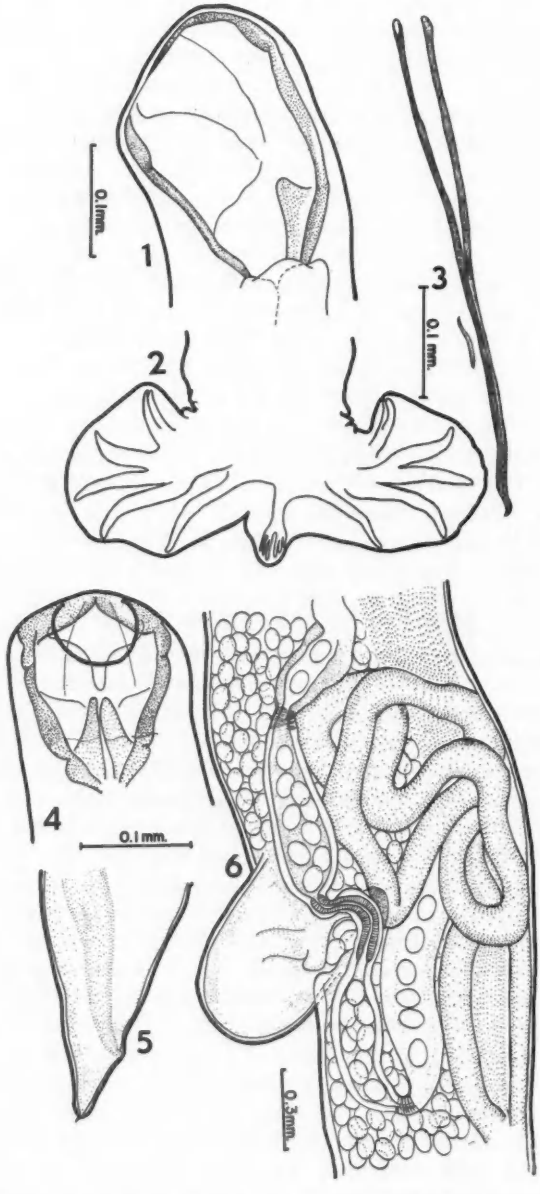

FIG. 1. Head, lateral view, female. FIG. 2. Tail of male. FIG. 3. Spicules and gubernaculum. FIG. 4. Head, dorsal view, male. FIG. 5. Tail, lateral view, female. FIG. 6. Vulva, female.

The vulva (Fig. 6) is located near the beginning of the posterior third of the female. In all specimens it possessed a prominent, salient, ventral flap which covers the vulva opening. The ovejector and vagina (Fig. 6) are typical. The eggs are oval (Fig. 6) with ample shells and measure 75 to 83μ by 60 to 68μ (measurements were made on eggs in the vagina and ovejector). The tail of the female is short (0.23 to 0.25 mm. long) and conical with a short spike at its tip (Fig. 5).

Type host: *Ursus americanus.*

Location: Small intestine.

Locality: Yukon Territory.

Type specimens: Deposited in the U.S. National Museum, Helminthological Collection, Beltsville, Md.

Discussion

D. yukonensis is similar to *D. stenocephala* (Railliet, 1884) Cameron, 1924, which is found typically in Canidae, and to *D. hamiltoni* (Baylis, 1933) n. comb., which is found in sea lions and elephant seals. *D. yukonensis* differs from *D. stenocephala* in being larger and having proportionally longer spicules. In having a salient vulva, *D. yukonensis* differs from specimens of *D. stenocephala* collected by the author from dogs in the Yukon, and specimens of this species and of "*U.*" *criniformis* from the U.S. National Museum. The specimens of *D. stenocephala* from dogs in the Yukon were smaller than the average size recorded for this species and had a salient excretory pore which *D. yukonensis* lacks. *D. yukonensis* differs from *D. hamiltoni* mainly on the basis of egg size, which Baylis (1) records as 135 to 138μ for the latter species. In other respects the two species are similar although *D. hamiltoni* comes from an unrelated host (pinniped) and geographical region (the Antarctic).

Hookworms have been reported from bears on several occasions. Stiles and Barker (9) list: *Ankylostoma malayanum* from *Helarctos malayanus* (Raffles); *A. brasiliense*, *A. caninum*, *A. ceylonicum*, and *A. malayanum* from *Melursus ursinus* (Shaw, 1791), and *Uncinaria ursi* (*ursimaritimi*) from *Thalarctos maritimus* (Phipps, 1774). The author has been unable to find further reference to hookworms in bears.

"*U.*" *ursi* (Duj., 1845) was described for females only and the description was so incomplete that this species must be considered a *nomem nudum*. *D. yukonensis* from *Ursus americanus* is considerably larger than *U. ursi* which is recorded as 6.6 to 7.6 mm. in length. The eggs, however, are similar in size, being 73 to 78μ for "*U.*" *ursi*. This species has not been referred to in the literature since the check list of Stiles and Barker (9) and that of Stiles and Hassall (10).

Biocca and Bronzini (2) described *D. thapari* new combination, from the panda, *Ailurus fulgens*, in the zoo of Rome. They place the host in the Ailurinae, family Ursidae. The pandas are more closely related to the raccoons than the true bears and belong to the family Procyonidae (8). The

general appearance of *D. thapari*, the shape of the buccal capsule, the lengths of the males and the females and of the spicules, and the size of the eggs suggest that it is similar to *Arthrocephalus lotoris* (Schwartz, 1925) Chandler, 1942 (4). *Arthrocephalus lotoris* (of Chandler) is closely related to *Dochmoides* differing from it mainly in the possession of distinct sutures between the scleratized buccal capsule plates.

The criteria used to separate species of *Dochmoides* are not clearly defined. Species which are closely related morphologically have been separated mainly by measurement differences. The author believes that measurements of body length are insufficient to separate species. Morphological characters are more satisfactory, and in support of them ecological, geographical, and host differences. The size of the egg in the female and the anatomy of the vulva seem to be good characters. In the male, the length of the spicules in proportion to body length and the shape of the gubernaculum are reliable. The shape of the buccal capsule and the form of the bursal rays have not been considered valuable by Baylis (1), Fülleborn (5), and Cameron (3). Although *Dochmoides* is closely allied with *Ancylostoma*, the latter genus has a complex buccal capsule which allows for its greater use in specific determination.

Host specificity is difficult to assess in the absence of experimental evidence. Baylis (1), in examining the "uncinarias" of badgers and canines found no difference between them, although Ransom (7), who worked with similar material before him, felt that he could distinguish between them. More important is the ubiquitous distribution of *D. stenocephala*, which has been reported from canines in all areas where other species of "*Uncinaria*" have been reported. For this reason eventually it may be proved that some species now separated from *D. stenocephala* on other than distinct morphological grounds are actually synonyms of it.

"*Uncinaria*" *lotoris* Schwartz, 1925 was described from raccoons near Baltimore, Maryland. Chandler (4) recovered specimens of this species from raccoons in Texas and determined that as he could see distinct sutures in the buccal capsule, this species was not an *Uncinaria* and made it a new combination with *Arthrocephalus* Ortlepp, 1925. He amended the diagnosis given by Ortlepp for this genus, dropping: "The absence of ventral lancets, presence of a dorsal cone, and longitudinal arrangement of female genital organs."

In the author's opinion, *A. gambiensis* Ortlepp, 1925, the type, and *A. lotoris* (Schwartz, 1925) are no more closely related than are species of *Ancylostoma* and species of *Necator*. The presence or absence of a dorsal cone is a generic character and Looss (6) places great reliance on the arrangement of the female genitalia in Ancylostomes. *A. gambiensis* does not appear to be closely related to *Dochmoides* whereas *A. lotoris* definitely is. If *A. lotoris* and with it a species with which it may be conspecific (*D.* (*U.*) *maxillaris* (Molin)), are not species of *Dochmoides*, then they represent a genus closely related to it which is distinct from *Arthrocephalus*.

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DENDROUTERINA PILHERODIAE SP. NOV. (DILEPIDIDAE) FROM PILHERODIAS PILEATUS (BODD.)[1]

By June Mahon[2]

Abstract

Dendrouterina pilherodiae sp. nov., Dilepididae, from a ciconiiforme bird from Brazil is described and the genus examined and redefined.

A vial containing several portions of tapeworm was forwarded to the Institute of Parasitology by Dr. Ernst Mayr, Museum of Comparative Zoology, Harvard College, who received the material from Dr. Helmut Sick, of the Fundação Brasil Central. This specimen proved to be a new species. The host names were checked according to Peters (8).

The drawings were made with the aid of a camera lucida.

The slides on which the following description is based will be deposited in the helminthological collection of the U.S. National Museum.

Dilepididae Fuhrmann, 1907: Dilepidinae Fuhrmann, 1907

Dendrouterina pilherodiae sp. nov.

Host: *Pilherodias pileatus* (Bodd.) (Ciconiiformes)

Locality: Brazil

There were several portions of strobila, one bearing a scolex.

The worm has a total length of about 60 mm. and a maximum breadth of 1.4 mm. The genital pores are unilateral, and open anteriorly on the lateral margin of the segment. All the segments are wider than long except the most gravid ones which are as long as they are wide. Large calcareous corpuscles are present in the parenchyma.

The scolex is small, diameter 90 μ mounted in Canada balsam, and is provided with four oval suckers, measuring 69 μ by 47 μ (Fig. 1). The rostellum is large and conical in shape. It has a diameter of 64 μ and a length of 76 μ. Only four hooks (Fig. 2) remain on the rostellum. They are all the same size and shape and measure 40 μ by 27 μ. By their shape, these would seem to be the large hooks.

Behind the scolex, the strobila narrows to form a short neck.

The excretory system consists of two pairs of longitudinal canals and a transverse commissure. On the poral side, the canals show the normal arrangement, a narrow dorsal vessel and a wider ventral one. On the aporal side the relative position of the narrow and wide vessels is reversed, and the wide canal is dorsal to the narrow one. In each segment the wide, ventral vessels are connected by a posterior, transverse commissure.

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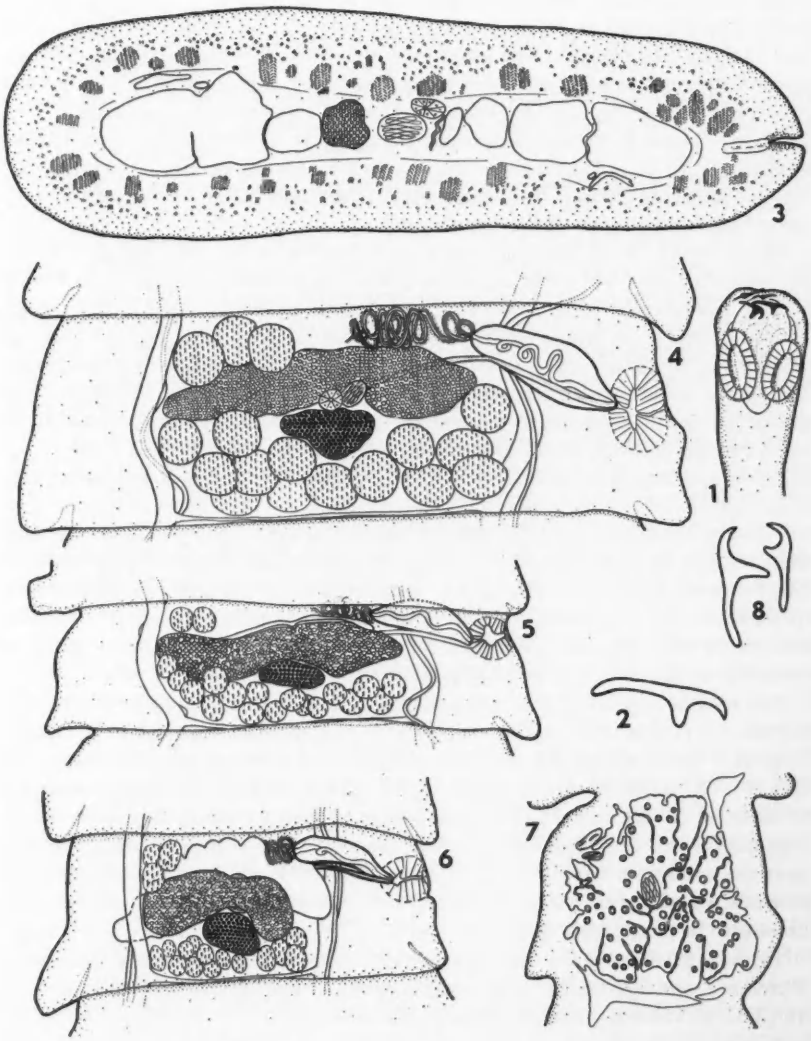
Contribution from the Institute of Parasitology, McGill University, Macdonald College P. O., Quebec, Canada, with financial assistance from the National Research Council of Canada.
[2]*Junior Research Assistant.*



FIGS. 1-7. *Dendrouterina pilherodiae.*

FIG. 1. Scolex. FIG. 2. Rostellar hook. FIG. 3. Transverse section of ripening segment. FIG. 4. Dorsal view of mature segment. FIG. 5. Dorsal view of segment showing appearance of young uterus. FIG. 6. Dorsal view of segment showing development of uterus. FIG. 7. Longitudinal section of gravid segment. FIG. 8. *D. botauri.* Rostellar hooks (after Rausch (10)).

The muscular system is well-developed and the usual elements are present, that is, longitudinal, transverse, and dorsoventral fibers. The longitudinal cortical musculature is made up of large fibers, but it is not well-organized into two definite bands. The outer circumference is composed of numerous, isolated fibers, which do not form a marked layer, and some of which extend out to the subcuticle. In the inner part, 10 to 20 fibers may be grouped into bundles. These large bundles occur particularly in the lateral part of the segment (Fig. 3). Transverse fibers separate the cortex from the medulla, and the latter is crossed by dorsoventral fibers which may extend almost to the subcuticular region of the cortex.

There are 15 to 20 testes per segment. The majority are situated behind the female organs, but three or four are lateral and even anterior to the ovary on the aporal side (Fig. 4). The vas deferens is very convoluted before entering the cirrus pouch, and forms no external seminal vesicle. The vas deferens is enveloped in a coat of cells which are glandular in appearance. The cirrus pouch, which measures 233 to 248 μ by 58 to 65 μ, is thin-walled, not very muscular, and extends medially past the poral excretory vessels. It contains the coiled cirrus; there is no internal seminal vesicle. The cirrus pouch opens into a large, well-developed, muscular, unspined, genital atrium (Fig. 4).

The vagina opens from the genital atrium dorsal to the cirrus pouch, and then swings posterior to the latter. The genital ducts pass dorsal to both poral excretory vessels. The vagina opens into a receptaculum seminis, which increases in size as the segments develop. At its maximum extent, the ovary reaches laterally almost to the excretory canals (Fig. 4). The vitelline gland is posterior to the ovary; the Mehlis' gland is well-developed.

The uterus appears earlier than is usual in the development of the female organs and is first seen as a transverse tube, anterior and ventral to the ovary (Fig. 5). As it increases in size, the uterus becomes lobed and filled with eggs (Fig. 6). Concurrent with the development of the uterus, the ovary continues to increase in size, until the enlarging uterus occupies more and more of the segment to the exclusion of the other organs. The uterus extends laterally past the excretory vessels on both sides, passing dorsal to them on the poral side and ventrally on the aporal side. The uterus remains lobed, even in the most gravid proglottids (Fig. 7).

In the ripest segments, the eggs are embryonated and possess six hooklets. There are two egg shells, both very delicate. The eggs have a diameter of 40 to 50 μ, and the embryos measure 22 to 25 μ.

Discussion

The type species is *Dendrouterina herodiae* Fuhrmann, 1912, from *Egretta garzetta garzetta* (L.) (Ciconiiformes) from Africa. Five other species have been described as belonging to this genus, namely:

D. fovea Meggitt, 1933 from *Dendrocitta rufa* (Lath.) (Passeriformes) from the Calcutta Zoo.

D. nycticoracis Olsen, 1937 from *Nycticorax nycticorax hoactli* (Ciconiiformes) from Minnesota, U.S.A.

D. lintoni Olsen, 1937 from *Butorides virescens* (L.) (Ciconiiformes) from Massachusetts, U.S.A.

D. botauri Rausch, 1948 from *Botaurus lentiginosus* (Montagu) (Ciconiiformes) from Wisconsin, U.S.A., and from *Ixobrychus e. exilis* (Gm.) (Ciconiiformes) from Ohio, U.S.A.

The type species lacked a scolex. The generic diagnosis given by Fuhrmann (3) was:

"Dilepidines with scolex unknown. Genital pores unilateral. Genital ducts pass between excretory vessels. Numerous testes behind the female genital organs. Uterus horseshoe shaped, with numerous lateral ramifications, extending above the excretory vessels on the poral side and below on the aporal side. The wide excretory vessel on the aporal side is dorsal to the narrow vessel. Parasites of birds. Type species *D. herodiae* Fuhrm., 1912."

Rausch (9) recorded that in the type specimen of *D. herodiae* the genital ducts actually pass dorsal to the excretory vessels and a year later (10), having examined specimens of *D. botauri* with scolices, he emended the generic diagnosis. Rausch (9) considers the original description of *D. fovea* Meggitt, 1933 (5) to be inadequate. This species is reported from a passeriform, whereas the genus *Dendrouterina* is characteristic of the Ciconiiformes, and the writer agrees that it is unlikely that the worm is in fact a *Dendrouterina*.

After examining whole mounts of the type specimens, Rausch (9) is of the opinion that *D. lintoni* and *D. nycticoracis* should be excluded from the genus *Dendrouterina*. For both of his species, Olsen (6, Fig. 12; and 7, Fig. 26) figures the inverted position of the aporal excretory vessels, but the uteri are not shown as being highly branched. The genital ducts pass between the excretory vessels. Coil (2) places these two species in the genus *Ophiovalipora* Hsü, 1935. Since the position of the genital ducts relative to the excretory vessels and the type of uterus clearly exclude these two species from the genus *Dendrouterina*, their measurements are not included in the table.

In 1955, Rausch (11) described a new species *Cyclustera ardeae* from *Ardea herodias* L. from Wisconsin, and proposed that *Dendrouterina* Fuhrmann, 1912 become a synonym of *Cyclustera* Fuhrmann, 1901. In the discussion the close similarities between the species *herodiae*, *botauri*, and *ardeae* are pointed out. These three forms show the distinctive features of the genus *Dendrouterina* as envisaged by Fuhrmann (4), namely the inversion of the aporal excretory vessels, the inverted U-shaped, gravid uterus with numerous lateral branches, and the relative position of this organ to the excretory vessels. In *herodiae* and *ardeae* the young uterus appears as a transverse tube, and in *D. botauri* it appears as a spherical sac, later forming a ring.

In the original description of *Cyclustera fuhrmanni* Clerc, 1906 (1) it is stated that there is only one pair of wide, longitudinal excretory vessels present, presumably the ventral ones, to which the genital ducts pass dorsally.

TABLE I

MEASUREMENTS OF *Dendrouterina* SPECIES

Species	*D. herodiae* Fuhrmann, 1912	*D. ardeae* (Rausch, 1955)	*D. botauri* Rausch, 1948	*D. pilherodiae*
Described by	Fuhrmann, 1912	Rausch, 1955	Rausch, 1948-49	Personal
Length	50–60 mm.	100 mm.	150 mm.	60 mm.
Breadth	2 mm.	2 mm.	2 mm.	1.4 mm.
Scolex	—	300 μ	165 μ	90 μ
Rostellum	—	—	—	64 μ
Suckers	—	—	65 μ	69 × 47 μ
No. of hooks	—	30	18	4
Large hook	—	43 μ	28 μ	40 × 27 μ
Small hook	—	33 μ	20 μ	—
Testes	44 in L.S.	40–65	45–56	15–20
Cirrus pouch	280–320 μ	130–135 × 60 μ	254–380 μ	233–248 × 58–65 μ
Eggs	20 μ	40 μ	33–40 μ	40–50 μ
Embryos	—	—	25 μ	22–25 μ
Host	*Egretta garzetta garzetta* (L.)	*Ardea herodias* L.	*Botaurus lentiginosus* (Montagu)	*Pilherodias pileatus* (Bodd.)
Locality	Africa	Wisconsin	Wisconsin *Ixobrychus e. exilis* (Gm.) Ohio	Brazil

Abbreviations used in table: Breadth: maximum breadth of specimens. Cirrus pouch: dimensions of cirrus pouch. Eggs: diameter of eggs. Embryo: diameter of embryo. Length: maximum length of specimens. No.: number. Testes: number of testes per segment.

In the redescription of *C. capito* (Rudolphi, 1819) Fuhrmann, 1909 (3) makes no mention of the excretory system, but the figure shows the normal arrangement of the aporal canals. In both, the ovary is deeply lobed.

In neither of these species is the gravid uterus described as extending above the poral excretory vessels and below the aporal ones, nor as being so highly branched.

In view of this, while cognizant of the ring-shape of the young uterus in *D. botauri*, the writer is not at present prepared to accept that *Cyclustera* is identical with *Dendrouterina*, and thus retains the use of the latter name. The measurements for the species *ardeae* are included in the table, since it obviously belongs to the same group.

The generic diagnosis of *Dendrouterina* Fuhrmann, 1912 would now read:

> "Dilepidinae: scolex well developed, rostellum armed with two rows of hooks. Genital pores unilateral. Genital ducts dorsal to longitudinal excretory canals. Testes numerous, the majority situated behind the female genital organs. Uterus horseshoe-shaped with numerous lateral branches extending above the excretory vessels on the poral side and below on the aporal side. The wide excretory vessel on the aporal side is dorsal to the narrow vessel. Parasites of birds. Type species *D. herodiae* Fuhrmann, 1912."

As may be seen from the table, *D. pilherodiae* differs from *D. botauri* in the size of the large hooks, and also in their shape (Figs. 2, 8). The large hooks of *D. pilherodiae*, while approaching in size those of *D. ardeae*, differ in shape. In *D. pilherodiae*, the cirrus pouch is smaller than in *D. herodiae* or *D. botauri* and is considerably larger than *D. ardeae*. The number of testes seen by Fuhrmann in a longitudinal section of a mature segment of *D. herodiae* is 44, and this figure is approached by the 45–56 for *D. botauri* and the 40–65 for *D. ardeae*. The number of testes in *D. pilherodiae* is considerably fewer. *D. pilherodiae* is the first member of this genus to be reported from South America.

Because of the persistent uterus, this genus falls into the subfamily Dilepidinae Fuhrmann, 1907.

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ASCAROID NEMATODES FROM CANADIAN BIRDS[1]

By Patricia M. Mawson[2, 3]

Abstract

Thirteen species of nematodes belonging to the superfamily Ascaroidea are recorded from birds from Canada. Of these, *Porrocaecum trichuriforme*, *Contracaecum anasi*, and *Heterakis pedioecetes* are described as new: *Contracaecum yamaguti* is a new name for *Contracaecum* sp. Yamaguti, 1941. Several stages in the life cycle of *P. ensicaudatum* are described and *Spiroptera turdi* Molin is regarded as a larval stage of this species.

The author had at her disposal a large collection of nematodes which had been taken from birds during the past 20 years by collectors for and members of the staff of the Institute of Parasitology at Macdonald College, Que. All birds were taken in Canada, but as many were sent in from various parts of the Dominion, the exact locality is not always known; where it is known it is given. The name of the host has been brought into line with those in the most recent North American check list.

The author wishes to express her gratitude to the Director and staff of the Institute of Parasitology for their help during this work.

The present paper deals with those members of the superfamily Ascaroidea found in the collection. Other major groups will be described in subsequent papers.

Porrocaecum ensicaudatum (Zeder)

(Figs. 1–9)

From the robin, *Turdus m. migratorius*, Macdonald College, Que. (lining gut and intestine),

the cowbird, *Molothrus a. ater*, Macdonald College, Que.,

the grackle, *Quiscalus quiscula*, Macdonald College, Que., intestine, loc. inq. (wall of duodenum),

the crow, *Corvus b. brachyrhynchus*, loc. inq.

The specimens from the cowbird and the crow are all adult females; they agree with the females from the robin in all respects. Those from the grackle are immature specimens, one a very young male, from the wall of the duodenum. From the robin the material falls into three age groups quite easily distinguished from one another but obviously belonging to the same species.

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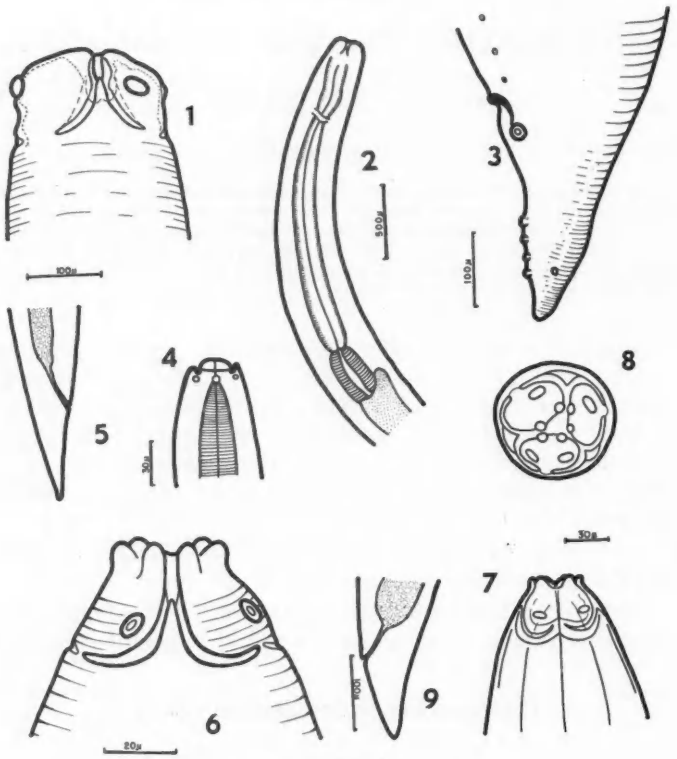


FIGS. 1-9. *Porrocaecum ensicaudatum.*

FIG. 1. Head. FIG. 2. Anterior end of adult. FIG. 3. Male tail of adult. FIG. 4. Anterior end of group 1 larva. FIG. 5. Tail of group 1 larva. FIG. 6. Head, lateral view. FIG. 7. Head, dorsal view. FIG. 8. Head, en face view of group 2 larva. FIG. 9. Tail of group 2 larva.

(1) Most of these specimens were from under the lining of the gizzard, but some were in the intestine with specimens of group 2. The worms are very immature; the head shows no resemblance to that of a heterocheilid worm; it is dome-shaped with a small terminal mouth and is surrounded by a strongly cuticularized collar apparently formed by the invaginated cuticle. Just posterior to the collar is a rim of four small submedian and two large lateral cephalic papillae. The oesophagus in a worm 4.3 mm. long and 160 μ wide, is 60 μ long, the ventriculus 140 μ long and 80 μ wide, the intestinal caecum 30 μ long, and the conical tail 130 μ long, ending in a point.

(2) From the intestine of the host. In these specimens the oesophagus is as described above, but there is a differentiation in the head region. The three lips and the interlabia are distinct, the lips outlined posteriorly by deep grooves which almost meet posteriorly at the bases of the lips. The anterior end of each lip is prolonged into two large cuticularized bosses. On the dorsal

lip are two large papillae and on each ventral lip one papilla. The dimensions of this form are: length, 3.7–4.8 mm., breadth, 130–150 μ; oesophagus (including ventriculus), 530–800 μ long, ventriculus, 120–150 μ long, 70–80 μ wide; intestinal caecum, 30–40 μ long. In this form, as in group 1, the tail is conical but it ends in a rounded tip and tapers more rapidly than in the younger form. One specimen was seen in which the external appearance was that of group 1 but the cuticle of the tail had separated partly from an underlying cuticle as if in preparation for an ecdysis and the shape of the inner tail was that of group 2. In the specimens of group 2 no sexual differentiation had taken place.

(3) Adult form. These are distinguished from group 2 mainly by the presence of reproductive organs, and by a slightly different form of the lips. The labial teeth and the lateral flanges are well-developed and the posterior groove separating lip from nuchal region is relatively shorter. These adults agree very well with descriptions of *Porrocaecum ensicaudatum*; in no case are lateral alae present, a feature which distinguishes them from *P. brevispiculum* Webster from the same host species, *Turdus migratorius*, from Ithaca, U.S.A. The specimens vary greatly in length with age; the largest males reach 35 mm., the females 59 mm. The dimensions in these longest specimens are: length of the oesophagus in male, 2.5 mm., in female, 3.3 mm., including the ventriculus which is 400 μ by 290 μ in the male and 550 μ by 340 μ in the female; the caecum is 110 μ in the male, 210 μ in the female; the eggs are 80–90 μ by 55–60 μ. The vulva is 1/2.3 of the body length from the head. The spicules are equal, 480 μ to 500 μ long. In all three groups the excretory pore is just behind the nerve ring.

The specimens from the wall of the duodenum of the grackle are young adults; the form of the head agrees with the characters of *P. ensicaudatum*. The posterior end of one male is present, in which the spicules, 0.16 mm. long, are hardly discernible, and the caudal papillae are arranged as in *P. ensicaudatum* on one side but are asymmetrical on the other. The only whole specimen among these is a female, 16.8 mm. long, with oesophagus 1.5 mm. long, including the ventriculus, 0.3 mm. long. The intestinal caecum is 100 μ long.

As far as is known to the writer no work has been done on the life history of *Porrocaecum* spp. (except on those parasites of fish and marine mammals now regarded as *Terranova* spp.). The apparent absence of a boring tooth in these larvae is of interest. The robins in the locality from which these specimens came apparently feed largely upon earthworms; the examination of a number of these however has failed to reveal larval *Porrocaecum* spp.

The figures and description of *Spiroptera turdi* Molin given by v. Linstow in 1884 (11) suggest that this species may be a young stage of a *Porrocaecum* sp. The earlier descriptions, of Molin, v. Drasche, and Diesing, were of sexually immature forms. v. Linstow found one male worm under the lining of the gizzard (*zwischen den Magenhauten*) of *Turdus pilaris*. He describes

this worm as having a glandular body between the intestine and the oesophagus; this is about a seventh of the length of the oesophagus and is presumably the ventriculus. The tail of this male is papillated very much as is *P. ensicaudatum*.

Two of v. Linstow's species, which he later regarded as synonyms of *S. turdi*, resemble different stages described above from the Canadian material. *Ascaris fissilabium* Linst. is like group 2 above; it came from the stomach of *Sturnus vulgaris*.

Filaria turdi v. Linstow (10) was taken from the "stomach" (? gizzard) wall, where it occurred in great numbers in the process of exsheathing. The figure and description agree very well with those of group 1 above even to the presence of an end bulb (ventriculus) on the oesophagus. v. Linstow refers to the rounded head with two projections; from the figure these are seen to correspond to the profile view of the collar described in the Canadian material.

This interpretation was also made of v. Linstow's figure by v. Drasche (4) who described *Spiroptera turdi* Molin as having a ring-like five-angled welt (Wülste). From his figure, however, it seems likely that v. Drasche was dealing with a slightly different stage, and that the welt which he describes may be the grooves around the bases of the lips.

v. Linstow in 1887 (12) also described what he considered part of the life history of this species. He found the adult under the lining of the gizzard, and larval stages in the intestine, with their heads and the anterior end of the body buried in the intestinal mucosa, and assumed that they were on their way to the gizzard lining via the intestinal wall. As v. Linstow referred to no other adult than the single male from *Turdus pilaris* (11), the assumption that this is the usual site of the adult is not entirely justified. It has been noted above that a very young male *P. ensicaudatum* was found in the wall of the duodenum of a grackle.

The writer has not herself dissected any of this species from their hosts, and has to rely on the labels in bottles. In the absence of any accurate knowledge of the location of the worms in the body and of opportunity for any experimental work on the life history, it can only be suggested that the early stage larva is ingested by the host, probably in an intermediate host such as an insect or an earthworm and undergoes some period of migration in the wall of the stomach and possibly in other organs, the young adult being usually encountered in the intestine.

Cori (2) described *S. turdi* Rud. from the blood vessels of earthworms in Central Europe.

About 20 earthworms from feeding grounds of robins at Macdonald College have been dissected. No nematodes were present in the blood vessels, but larval nematodes were found in the septa (? *Syngamus* sp.) and in cysts which were often grouped together surrounded by a dark brown granular material (? *Rhabditis* sp. larvae).

Some of both kinds of larvae were fed to chickens but after three weeks the chickens were not infected with any nematodes.

Porrocaecum trichuriforme n. sp.

(Figs. 10-13)

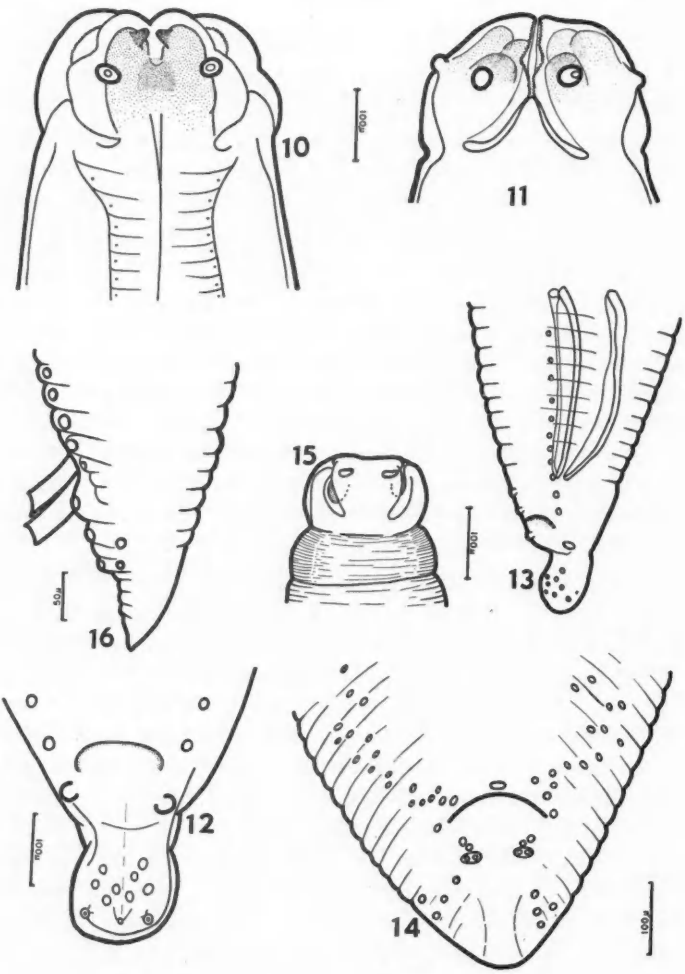
From the great horned owl, *Bubo v. virginianus*, loc. inq.,
an "owl", sp. et loc. inq.,
duck hawk, *Falco peregrinus anatum*, Dundas Harbour, proventriculus.

The females from the owls measure up to 120 mm., the males to 100 mm. in length. The form of the adult worm is distinctly stouter in the posterior quarter or third. The specimens from the duck hawk are young females, one in the process of exsheathing, and they are 43 to 83 mm. long.

The lips have well-developed lateral flanges on to which the denticular ridge is continued. The pulp of the lips forms two large lateral lobes and a median lobe internal to these. The triangular interlabia are very short, about a third the length of the lips. In a female 120 mm. long the oesophagus is 2.7 mm. long, slightly wider in its second half, the small almost spherical ventriculus is 0.3 mm. long, and the intestinal caecum 1 mm. long. The nerve ring is 0.7 mm. from the head. The vulva is very small and insignificant, and lies at the end of a long thin vagina. It is at the end of the first quarter of the body length from the tail, and all ovaries and uteri are posterior to this. The tail of the female is short and rounded.

In the male the reproductive organs all lie in the posterior third of the body. The tail narrows suddenly at about its midlength, the second half being rounded and knob-like. There is a pair of large single papillae just behind the anus, five pairs of papillae, not very symmetrically arranged, on the narrow part of the tail, and one small median papilla near the end of the tail. There are at least 20 pairs of preanal papillae. The alate spicules are 0.64 mm. long.

P. depressum is the common parasite in birds of prey. It has often been recorded but has not been adequately described. Recently Morgan and Schiller (14) have given a fuller description than has been available. As the specimens described above differ in several points from this and from that given by Baylis (1), they are regarded as belonging to a new species. The features which distinguish this species from *P. depressum* are the shortness of the ventriculus as compared with the oesophagus, the shorter intestinal caecum, and the difference in shape of the labial pulp. This last is described briefly but not figured by Morgan and Schiller, and it is presumed that in their material it agreed with that figured by Baylis (1). The position of the vulva is not clearly indicated by Morgan and Schiller, who give the length of the females as from 30–120 mm. and the distance of the vulva from the head as from 24–36 mm., a very variable position in terms of the quarters of the body length of the female. The vulva is said by Baylis to lie a little in front of the midlength.



FIGS. 10-13. *Porrocaecum trichuriforme* n. sp.

FIG. 10. Head, dorsal view. FIG. 11. Head, lateral view. FIG. 12. Male tail, ventral view. FIG. 13. Male tail, lateral view.

FIG. 14. *Anisakis diomedeae.* Male tail, ventral view.

FIG. 15. *Contracaecum magnicollare.* Head, dorsal view.

FIG. 16. *C. podicipitis.* Male, tail, lateral view.

Contracaecum magnicollare Johnston and Mawson

(Fig. 15)

From an albatross, *Diomedea* sp., Goose Is., B.C.

Two male worms are present. This species has been recorded in the southern hemisphere from *Anous stolidus* and *Diomedea cauta** from Australia, and from *Sula serrator* from New Zealand (5, 6, 7).

The measurements of the two male worms are: length 16.3, 20 mm., oesophagus 1/7.1, 1/8.3, and spicules 1/3.3, 1/3.7, of the body length; oesophageal caecum and intestinal caecum respectively 1/2.7, 1/3 and 1/1.3, 1/1.4, of the length of the oesophagus. In these specimens the head can be seen in better view than was the case with the original, and a figure is given of it.

Contracaecum podicipitis Johnston and Mawson, 1949

(Fig. 16)

From the pied billed grebe, *Podilymbus p. podiceps*, loc. inq.

The characters of the head agree perfectly with the description of the type which was from an Australian grebe, *Podiceps cristatus*. The spicules are a little shorter and the oesophageal appendix a little longer, but the variation is not great. In describing the type, it was not possible to get a clear picture of the male tail, on which only two pairs of postanal papillae could be seen. In the present specimen these are very clear. There is on each side of the tail just behind the anus, one large papilla and behind this four smaller ones, all on the proximal half of the tail. One male and one female worm are present. The length of the male is 19.2 mm., the oesophagus is 1/5.6 of the body length, the oesophageal appendix is 1/2.3, and the intestinal caecum is 1/1.4 of the length of the oesophagus; the spicules are 2.7 mm. long, 1/7.1 of the body length. The vulva in the female is in the third 1/7 of the body length. The eggs are 55–60 μ in diameter.

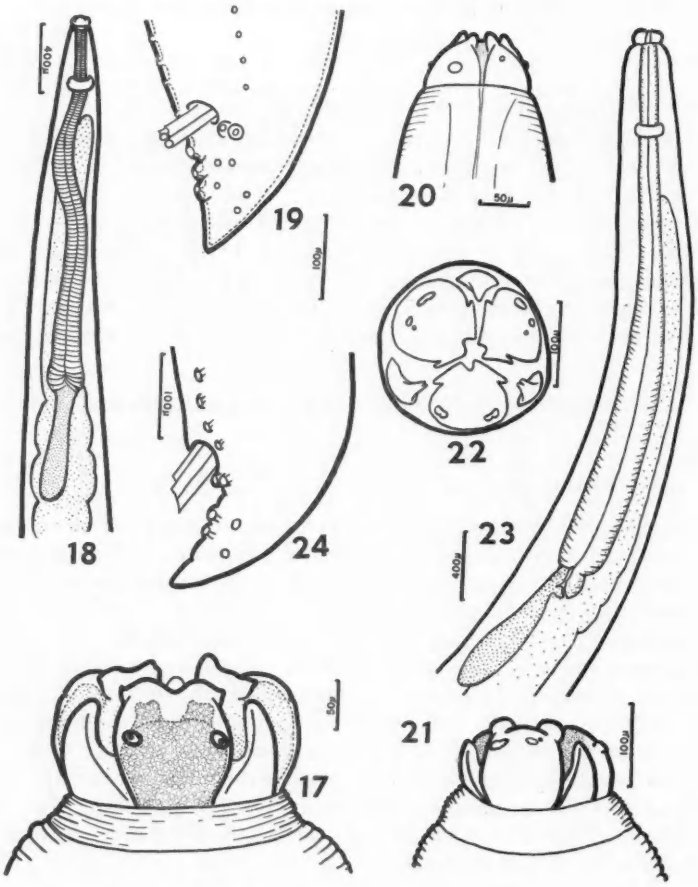
Contracaecum anasi n. sp.

(Figs. 17-20)

From the black duck, *Anas rubripes*, Cape Smith, Ungava.

The material available consists mostly of young worms, four of which are in the phocascarid larval stage, two are exsheathing from this stage, three are young females, one an older female, and one a young male. There is also the tail of an older male and the greater part of an older female containing eggs. A table comparing the measurements of these forms is given; though few specimens are present, the measurements indicate that the oesophageal appendix and oesophagus do not increase in length at the same rate as the body, or as each other.

* *Not Diomedella cauta, a misprint in* (*8*).



FIGS. 17-20. *Contracaecum anasi* n. sp.
FIG. 17. Head, dorsal view. FIG. 18. Anterior end. FIG. 19. Male tail. FIG. 20. Phocascarid larva, head.
FIGS. 21-24. *Contracaecum yamaguti*, nom. nov.
FIG. 21. Head. FIG. 22. Head, en face. FIG. 23. Anterior end. FIG. 24. Male tail.

The head is slightly narrower than the body immediately following, and the collar is narrow. The interlabia are almost as long as the lips and some of them are bifid at the tips. The lips have small or no lateral flanges. The anterior end of each lip is deeply indented; the anterolateral projections are well-developed; the pulp projects anteriorly in two unbranched masses, more or less truncated anteriorly. The large papillae, two on the dorsal lip and one on each subventral lip, are single-headed; there are in addition on the subventral lips a small papilla and an amphid, situated close together rather

TABLE I

MEASUREMENTS OF *Contracaecum anasi* n. sp.

	Phocascarid larvae				Exsheathing		Young females			Adult female	Young male
Length	6.5	7.5	7.6	8.3	6.5	8.8	11.2	16.3	17.5	24.5	14.7
Oes/L	1/6.4	1/6	1/7.6	1/7	1/7.2	1/7	1/6.6	1/7.7	1/7.3	1/8	1/7
O.A./Oes	1/3	1/3.1	1/2.2	1/2.4	1/3	1/3.6	1/2.8	1/3.8	1/3.4	1/4	1/3.5
I.C./O	1/1.5	1/1.6	1/1.1	1/1.3	1/1.5	1/1.4	1/1.4	1/1.3	1/1.3	1/1.4	1/1.5

more anteriorly on the lip than the large papilla. In the phocascarid larvae the form of the lips, though they are smaller and less voluminous, resembles that of the adult; the interlabia are absent.

The small spherical ventriculus of the oesophagus is well-developed. The nerve ring is a quarter to a sixth of the length of the oesophagus from the head.

The spicules in the single specimen are very long; 1/3.3 of the body length. The seven pairs of papillae on the tail are arranged as in the figure; on each side two large papillae lie close together, behind these are two smaller ones not quite so close together, behind these is a single one and lateral and posterior to this are two others. Anteriorly there are at least 12 pairs of preanal papillae.

The vulva was seen in two specimens; it is relatively far forward at the end of the first quarter. The eggs are 50 μ by 60 μ.

The species is very close to *C. spiculigerum*, from which it differs in the arrangement of papillae on the male tail and in the narrowness of the collar.

Contracaecum yamaguti nom. nov.

Syn. *Contracaecum* sp. Yamaguti, 1941

(Figs. 21-24)

From *Mergus merganser americanus*, loc. inq.

Yamaguti (18) described fully a species from the Japanese merganser, but refrained from naming it as new as he had not been able to compare it with *C. turkestanicum* Skrjabin (16) from the same host species. Through the kindness of the United States Department of Agriculture this description is now available. *C. turkestanicum* is known only from larvae taken from under the lining of the gizzard of the host. It is represented by the same specimens as were described by Skrjabin (15), as *Contracaecum* sp., 1916. As far as can be seen from the larvae, they are not conspecific with the Japanese worms.

Specimens from the American merganser agree closely with the description given by Yamaguti (although he gives no figures). The name *C. yamaguti* is proposed. The chief measurements of the Canadian specimens are as follows: length male 30–36 mm., female 39–56 mm., young specimens not sexually

developed 27–30 mm. Oesophagus shorter than is usual in the genus, 1/10–1/11 of body length in both sexes and 1/9–1/10 in the young. Oesophageal appendix 1/4–1/4.2 in all groups. Spicules 1/6–1/6.6 of body length, except in one male where it is 1/8.

Contracaecum spiculigerum (Rudolphi)

From the crested cormorant, *Phalacrocorax a. auritus*, from Creesy Lake (St. Andrews), N.B.

A number of specimens of this common parasite of cormorants are present.

Contracaecum pelagicum Johnston and Mawson

From an albatross, *Diomedea* sp., Goose Is., B.C.

One young female only is present. It is referred to this species on account of the tooth-shaped prominences on the anterolateral projections on the lips, and because of the relatively short oesophagus. The species has been recorded from related birds from Australia (6) and (9). The present specimen is 24 mm. long, with oesophagus 1/9 of the body length.

Anisakis diomedeae (Linstow)

(Fig. 14)

From an albatross, *Diomedea* sp., Goose Is., B.C., throat and stomach.

A number of specimens of both sexes are present. Only one male of the species has been described before (8), and in that the spicules were so lightly chitinized that their length was not obtained. In the present specimens the cuticle on the posterior end of the body from a short distance in front of the anus, is raised in a series of small vesicles or blisters, apparently a natural condition, so that the caudal papillae are obscured. Of these, there are certainly four pairs immediately postanal, and four more laterally at about the midlength of the tail. There may be more. Two of the postanal pairs lie very close together and these were not seen in the male described by Johnston and Mawson. The spicules are acicular for the greater part of their length with a widened proximal end; they are 1.1 to 1.3 mm. long.

En face view of the head has established the presence of a small papilla in addition to the large double one on each subventral lip. The body of the fully adult female is widest in the posterior half, which is of even width and tapers in the anterior part. The vulva is just behind the middle of the body; the eggs are spherical and thin-shelled, with a diameter of about 40 μ.

The dimensions are as follows: length of male 35–44 mm., of female 36–51 mm.; oesophagus (excluding ventriculus) 2.9–3.4 mm. in male, 3.8–4.7 mm. in female; ventriculus 1.1–1.3 mm. in male, 1.5–1.7 in female. In the male the distance from the anterior end of the nerve ring is 0.14 mm., of the cervical papillae 0.5 mm. In two specimens a structure resembling the excretory duct and pore was seen at the level of the nerve ring.

Ascaridia bonasae Wehr, 1940

From the ruffed grouse, *Bonasa umbellus*, loc. inq., Hudson, Que., La Tuque, Que., La Tuque, Que., Pointe au Chêne, Que., Scotch Lake, N.B., Nipiquisiti R., N.B.,
"grouse", N.W.T.,
sooty grouse, *Dendragopus obscurus fuliginosis*, loc. inq.

The specimens agree with the description given by Wehr (17). In his description the egg size is not given; in the Canadian specimens this is 100 μ by 60 μ.

Ascaridia columbae (Gmelin)

From the domestic pigeons, *Columba livia*, Macdonald College, intestine.

This species has already been recorded from Canadian pigeons by M. J. Miller (13).

Heterakis pedioecetes n. sp.

(Figs. 25-26)

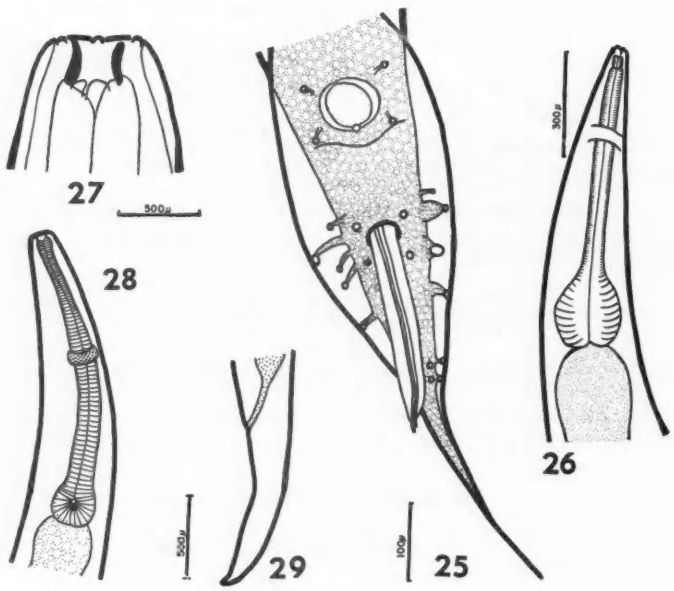
From the sharp-tailed grouse, *Pedioecetes p. phasianellus*, loc. inq.

The material consists of several males and females. They closely resemble *Heterakis bonasae* Cram (3) from *Bonasa umbellus* in the U.S.A. The main difference lies in the lengths of the spicules which in *H. bonasae* are stated to be almost equal in length (1.4–1.6 mm., 1.1–1.3 mm.) and in the Canadian material are definitely unequal (1.6–1.8 mm., 0.6–0.7 mm.). Through the courtesy of the Agricultural Research Service of the U.S. Department of Agriculture, it was possible to examine the type male of *H. bonasae*. Unfortunately, this has been made into a permanent mount and can only be viewed from the lateral aspect. One spicule (the longer) is clearly visible and the other may be directly behind it, but two heads cannot be distinguished. Examination of unmounted specimens in the future may show that the spicules of *H. bonasae* have been misrepresented, in which case the present species will fall as a synonym.

Other minor differences are present in the male tail, namely, the sucker is smaller (57 μ internal and 80 μ external diameter instead of 83 μ and 100–120 μ as in *H. bonasae*) and the tail is longer, as well as the spike being longer relative to the anterior part of the tail than in *H. bonasae*.

The arrangement of papillae in the male tail is like that of *H. bonasae* but there is a difference in the most posterior group of papillae, which in *H. bonasae* consists of three papillae on each side, and in the present species has four pair, or two pair.

The measurements of the male and female of the new species are as follows: length male 6.5–6.7 mm., of female 7.4–9.3 mm., breadth of male 320–350 μ,



FIGS. 25-26. *Heterakis pedioecetes.*
FIG. 25. Male tail. FIG. 26. Anterior end.
FIGS. 27-29. *Subulura* sp.
FIG. 27. Head. FIG. 28. Oesophagus. FIG. 29. Tail.

of female 240–430 μ; length of oesophagus (including bulb) 0.9 mm. in male, 0.98–1.1 mm. in female, and diameter of spherical bulb 150–200 μ in both sexes. Tail length in male 460–500 μ, with appendix 130–140 μ, and in female 1.1–1.2 mm. The vulva is just behind the midbody; the eggs are 60–65 μ by 39–40 μ.

Subulura sp.

(Figs. 27-29)

From the shoveller duck, *Spatula clypeata*, loc. inq.

One female only was present. The dimensions are as follows: length 26 mm., oesophagus 1.8 mm. long, buccal cavity 40 μ deep, 40 μ internal and 60 μ external diameter, with three small equal sized teeth at its base; distance of nerve ring from anterior end 0.8 mm.; eggs 80 μ by 53 μ; position of the vulva not seen.

To the writer's knowledge no species of the genus *Subulura* has yet been recorded from Anseriforme birds, but it is felt that this single female worm cannot be allotted to a species.

List of Parasites

Porrocaecum ensicaudatum (Zeder), *Turdus m. migratorius*, Que., *Molothrus a. ater*, Que.; *Quiscalus quiscula*, Que.; loc. inq.; *Corvus b. brachyrhynchos*, Que.; loc. inq.

Porrocaecum trichuriforme n. sp., *Bubo v. virginianus*, loc. inq.; *Falco peregrinus anatum*, Que.; "owl", loc. inq.

Contracaecum magnicollare, Johnston and Mawson, *Diomedea* sp., B.C.

Contracaecum podicipitis Johnston and Mawson, *Podilymbus p. podiceps*, loc. inq.

Contracaecum anasi n. sp., *Anas rubripes*, Ungava Peninsula.

Contracaecum yamaguti nom. nov., *Mergus merganser americanus*, loc. inq.

Contracaecum spiculigerum (Rudolphi), *Phalacrocorax a. auritus*, N.B.

Contracaecum pelagicum Johnston and Mawson, *Diomedea* sp., B.C.

Anisakis diomediae (Linstow), *Diomedea* sp., B.C.

Ascaridia bonasae Wehr, *Bonasa umbellus*, Que., N.B.; loc. inq.; *Dendragopus obscurus fuliginosus*, loc. inq.; grouse, N.W.T.

Ascaridia columbae (Gmelin), *Columba livia*, Que.

Heterakis pedioecetes n. sp., *Pedioecetes p. phasianellus*, loc. inq.

Subulura sp., *Spatula clypeata*, loc. inq.

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A METHOD FOR THE ASSESSMENT OF THE NUTRITIONAL STATUS OF WILD UNGULATES[1]

By P. J. Bandy,[2] I. McT. Cowan,[3] W. D. Kitts,[4] and A. J. Wood[5]

Abstract

A study has been made of the relationship existing between heart girth and body weight and hind foot length and body weight of growing Columbian black-tailed deer (*Odocoileus hemionus columbianus*). From the results obtained, a method has been proposed for the assessment of the nutritional status of wild ungulates. This method involves a comparison of ratios of body weight estimated from heart girth to body weight estimated from hind foot length.

Introduction

The evolution of a method for the assessment of the nutritional status of wild ungulate populations is one of the major prerequisites to the establishment of sound management procedures. Such a method should be based on measurements that can be readily obtained with a minimum of facilities under field conditions. The appraisal of range capacity by the measurement of forage density and its approximate composition has shed some light on the problems of individual and population growth (Cowan *et al.* (2); Einarsen (3); Nichol (6)). Leopold *et al.* (5) and Cheatum (1) have provided a means of estimating the starvation state in deer by examination of the bone marrow. This method has merit for assessment of acute malnutrition but unfortunately may not permit measurement of degrees of departure from a theoretical optimum.

In the course of our studies upon the influence of the plane of nutrition on the growth of the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) we have accumulated certain growth data which appear to afford a practical and valid technique for developing an index with which to express nutritional status. It has been found that actual body weight can be estimated with a reasonable degree of precision from heart girth measurements while the measurement of hind foot length permits a prediction of the ideal body weight under optimum conditions. The ratio of the two body weights for any given animal can then be used to describe the nutritional status of that animal.

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Contribution from the Department of Zoology and the Division of Animal Science, The University of British Columbia, Vancouver, B.C. Financial assistance was received from the National Research Council of Canada.
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Experimental

Groups of animals (male and female) have been reared from birth on high and low planes of nutrition. The maintenance procedures, nutritional regimen, and the methods used for the measurement of the various growth parameters will be described elsewhere. To facilitate comprehension of the present contribution it should be stated that (1) body weight was taken at weekly intervals, (2) heart girth was measured with a $\frac{3}{8}$ in. flexible steel tape around the chest immediately behind the axilla, and (3) hind foot length was taken from the tip of the hoof to the tip of the calcaneous with the deer in standing position.

Results and Discussion

Fig. 1 presents in graphic form the relationship that was found to exist between body weight and heart girth. The regression line ($M = 0.00037G^{2.64}$) was developed using some 225 measurements taken from 10 low plane animals and 10 high plane over a period of two years. It suggests that a 1% increase in the heart girth (G) of the growing animal is accompanied by a 2.64% increase in body mass (M). It is well to reiterate that this relationship represents the case of the growing animal. The case of the mature animal

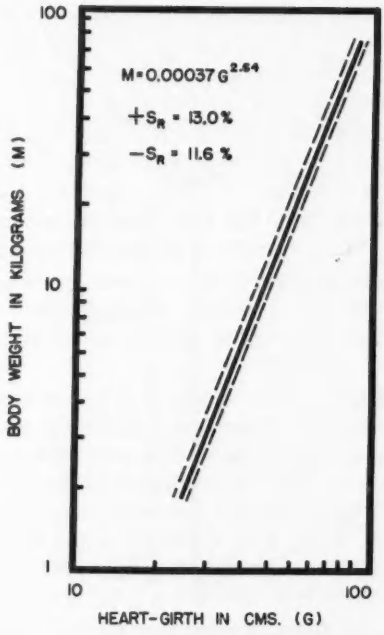

FIG. 1. The relationship between body weight and heart girth of the Columbian black-tailed deer.

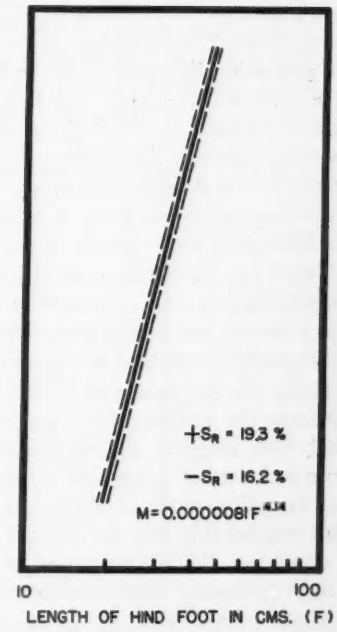

FIG. 2. The relationship between body weight and hind foot length of the Columbian black-tailed deer.

losing and gaining adipose tissue in response to varying planes of nutrition is at present under consideration. Heart girth, involving as it does measurements of the skeleton of the chest along with the overlying muscular and adipose tissue, provides an excellent indication of the bulk of the animal and thus its body weight. The heart girth increases and decreases in response to gain or loss in real body weight. It is relatively independent of "fill" in the gastrointestinal tract.

The length of the hind foot, on the other hand, is a measure of skeletal dimension and is therefore unchangeable once achieved. While it is impossible to state the energy requirement of skeletal growth it is safe to assume that the nutritional priority for the development of this structure is much higher than for the deposition of adipose tissue and perhaps also for extensive hyperplasia of muscle systems. Hammond's (4) work with sheep and swine certainly supports such a contention. Added to this relatively high priority for available nutrients is the established fact that in the deer a greater percentage (50%) of skeletal development has been achieved at birth than is the case for body weight (3%) and heart girth (26%). This means that the plane of nutrition subsequent to birth can have a much greater relative influence on body weight and heart girth than on the hind foot length. Fig. 2 presents the relationship which was found to exist between body weight and hind foot length of the high plane male deer. The regression line ($M = 0.0000081F^{4.14}$) indicates that a 1% increase in hind foot length (F) is associated with a 4.14% increase in body weight (M). In the case of low plane male deer the regression line was found to have the form represented by the equation $M = 0.000016F^{3.91}$. A comparison of the exponent (3.91) for these animals with that found for the high plane animals (4.14) indicates that the plane of nutrition has had little or no effect on the rate of development of hind foot length. The difference between the two exponents is not significant. For present purposes the body weights computed from the high plane regression line have been used since they will more nearly represent optimum conditions.

There can be little question that under conditions where a low plane of nutrition exists skeletal growth will be impaired; however, the proportionate effect on the two parameters of growth used here will be such as to influence heart girth to a greater relative extent.

Using the regression of hind foot length on body weight it is possible to estimate the weight any given animal should have achieved for its existing hind foot length. The heart-girth – body-weight regression will afford a close estimate of the actual body weight. A comparison between these two weights will provide an indication of the current nutritive state of the animal. This relationship may be expressed, $Ic = (WHg/WHf)$ where Ic is the index of condition, WHg is weight estimated from heart girth, and WHf is optimum weight estimated from hind foot length.

Table I illustrates the application of this index of condition derived from the growth data for two of our male deer. Series A relates the two animals at equal body weights. Under these circumstances the high plane animal is

TABLE I

THE INDEX OF CONDITION FOR DEER 1H AND 3H

Deer No.	Plane of nutrition	Actual wt., kgm.	Heart-girth, cm.	Est. wt. from heart girth	Length of hind foot, cm.	Est. wt. from hind foot	Index of condition
Series A							
1H	Low	34.04	75.5	34.5	42	36	0.96
3H	High	34.04	74.0	33.0	39	28	1.18
Series B							
1H	Low	32.67	75.0	34.0	42	36	0.95
3H	High	68.74	97.0	66.0	42	36	1.84

considerably younger than the low plane animal. The heart girths of the two animals are identical (within the error of measurement) and hence the body weights estimated from heart girth are identical. The hind foot length of the low plane animal is longer than that of the high plane since at equal actual body weight the low plane animal is appreciably older than its high plane counterpart. When weights are estimated from these hind foot lengths it is found that the high plane animal will yield a body weight below that computed from heart girth, while the low plane animal will yield a weight in excess of that obtained from the heart girth measurement. The index of condition is 0.96 for the low plane and 1.18 for the high plane. Series B represents the same two animals at equal hind foot lengths. At this time the

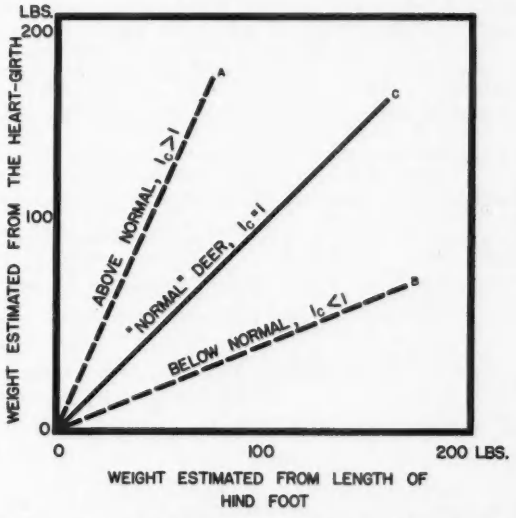

FIG. 3. A graphic expression for the index of condition.

high plane animal weighed twice as much as the other and the index of condition for the two, 0.95 for the low and 1.84 for the high, reflects this situation.

A graphic method can also be used in the evaluation of nutritive condition (Fig. 3). If the weight estimated from heart girth equals that estimated from hind foot length (normal deer) the index of condition will be 1.0. If the two estimates of weight are plotted against each other the normal deer will be represented by any point falling on the line *OC*. Deer in above normal condition will fall above this line and those suffering from the results of an inadequate dietary intake will fall below the line. It should be appreciated that an animal which has received a maximum caloric intake without adequate protein, calcium, or phosphorus will yield an index above one even though its growth rate may be subnormal in relation to its age. The likelihood of such a situation arising under field conditions seems most improbable.

The standards used in the proposed method of evaluating condition apply only to male Columbian black-tailed deer. The method is, however, applicable to other species for which the necessary reference bases can be established. Such reference bases could be constructed from field data but more satisfactory results would be obtained by using animals reared on a controlled feed intake under laboratory conditions.

The evaluation of range adequacy from the index of condition of animals taken on a particular range seems possible. The extension of the present work to large scale field studies is in process to determine the feasibility of the above proposal.

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SEASONAL CHANGES IN INSULATION OF THE FUR[1]

By J. S. Hart

Abstract

Seasonal changes in insulation of the fur have been compared in nine species of arctic and North temperate zone mammals. Relative seasonal changes ranged from 12 to 52% of the winter value. Absolute changes were greatest in the larger mammals with thickest fur. The limited seasonal insulative change in small mammals suggests that changes in heat production may be important in seasonal acclimatization.

Introduction

Since earliest times the role of fur as an insulator has been enhanced by casual observations of greater thickness in the winter months. Although seasonal change in fur is generally appreciated, only a few actual measurements of fur characteristics (3, 5, 7, 10, 13) have been made, and practically no data are available on seasonal changes in insulation.

Admittedly, measurements of insulation of the fur are somewhat arbitrary and do not give a complete picture of the seasonal change in over-all body insulation of an animal. They do nevertheless give useful information on the extent of the change in different species. The present survey of seasonal changes in various Canadian mammals is intended as a supplement to comparative information already available on insulation (1, 4, 6, 12) and as a contribution to knowledge on seasonal acclimatization in general.

Materials

Most of the pelts were obtained from the National Museum of Canada. Varying-hare pelts were obtained from the Royal Ontario Museum of Zoology. Muskrat, red fox, and red squirrel skins were contributed by the Canadian Wildlife Service. Species and localities are given below. Shortage of specimens precluded limitation of this study to a particular geographic area for most species.

Deer mouse (*Peromyscus maniculatus*): Ottawa area.

Brown lemming (*Dicrostonyx groenlandicus*): Mackenzie River District, Tavane, Hess Lake, Kazaan River, N.W.T.

Red squirrel (*Sciurus hudsonicus*): Fort Smith area, N.W.T.

Muskrat (*Ondatra Bibethica*): Fort Smith area, N.W.T.

Varying hare (*Lepus americanus*): most from Sudbury, Ontario; two from Nova Scotia.

Red fox (*Vulpes fulva*): mostly from Fort Smith, N.W.T.; two winter pelts from Prince Albert Park, Sask.

Wolverine (*Gulo luscus*): Coronation Gulf, Artillery Lake, and Barry Island, N.W.T.

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Wolf (*Cannis lupus*): Banks Island, Point Comfort, Coronation Gulf, Artillery Lake, Henderson Strait area, N.W.T.; others from Prince Albert Park, Sask.; and Pontiac County, Que.

Black bear (*Euarctos americanus*): Yukon; Yoho Park, B.C.; Waterton Park, Alta.; Vancouver Is., B.C.; Island Lake, Sask.

Polar bear (*Thalarctos maritimus*): Cape Bathurst, Banks Island, Smith Sound, N.W.T.; and Bear Island (James Bay).

Furs in prime winter condition were from animals caught during the period November–March. Summer pelts were from animals caught from May to September. Some of the pelts had been in storage since 1910, but they all appeared to be in very good condition.

Methods

Conventional hot plate and guard ring apparatus was used in this study. Two sizes of hot plate 10 in. × 10 in. and 3 in. × 3 in. were employed. The smaller instrument was similar in design to the larger one, which has been previously described (9). The latter was made available by Dr. Niven, Division of Applied Physics, N.R.C. Mouse pelts were tested on a cylindrical apparatus previously described (6).

Before measurement, single skins were mounted on a frame that was secured to the hot plate apparatus. To ensure close contact with skin and plate, the hide was wetted and stretched slightly before it was placed in the frame. The fur was fluffed up to give maximum insulation after the apparatus with skin was placed in a constant temperature room, away from drafts. The temperature difference from plate to air was recorded until thermal equilibrium was established, which required up to 24 hr. with heavy skins. Insulation was calculated in the conventional manner from the power input, temperature difference, and area.

Fur thickness measurements were also carried out on some pelts. Conventional methods of measuring pile fabrics employ the compression principle. However, most devices used for this purpose compress the relatively thin fur of animals too much. Therefore, a light piece of metal (0.35 gm./cm.^{2}) was placed on the fur and the average distance between the hide and metal under surface was taken as the fur thickness. No insulation measurements were made on lemmings; results were estimated from fur thickness measurements.

Results and Discussion

There were substantial seasonal changes in insulation and thickness of the fur in the larger mammals (Fig. 1). In general, change in fur depth seemed to be the main factor affecting the change in insulation since summer and winter furs showed approximately the same correlation between thickness and insulation. Species differences in insulation for a given thickness were nevertheless apparent, as previously observed by others (4, 12). In both seasons wolf skins showed the greatest insulation for their depth, while polar bear skins showed the lowest insulation, with wolverine fur intermediate. The relatively

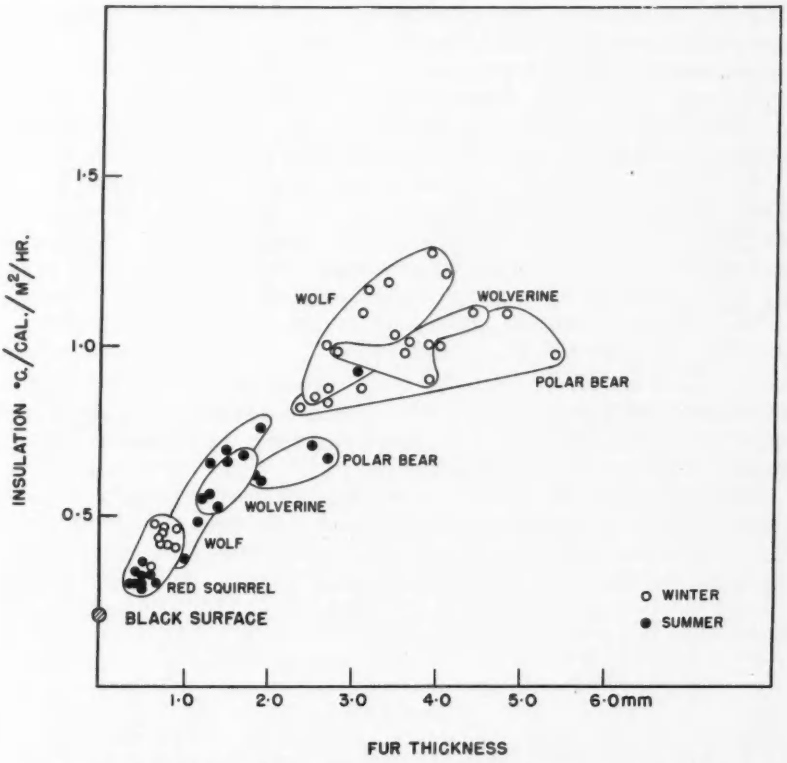

FIG. 1. Insulation of the fur in relation to fur depth in summer and winter.

coarse hair of the polar bear has been previously noted (12). The method used to measure fur thickness appears to have no advantage over that employed by Scholander *et al.*, judging from the variability of the data.

Table I shows the mean value for summer and winter insulation and the relative changes, which ranged from 12–52% of the winter values. Unlike the absolute changes, relative change in insulation of the smaller mammals was not markedly inferior to that of the larger mammals. The varying hare and the muskrat showed the smallest relative change. The slight seasonal difference of the muskrat is especially interesting in view of its aquatic habit. Like the beaver (12), the fur of this species is adapted to heat conservation in water. The short fur with dense, woolly under hair is wetted to only a slight extent by water, leaving an insulating layer of air in the fur when the animal submerges.

The correlation between insulation and body weight (Fig. 2) shows the main trends and individual data. Vertical bars show results obtained by Scholander *et al.* (12) for winter pelts of five of the species studied. Good agreement with Scholander's data was found for all except the varying hare,

TABLE I

SEASONAL CHANGE IN INSULATION

Species	Insulation, ° C./cal./m.²/hr.		Seasonal change, % of winter value
	Summer	Winter	
Deer mouse	0.180	0.229	21.4
Red squirrel	0.316	0.438	29.2
Muskrat	0.534	0.606	11.9
Hare	0.489	0.582	16.0
Red fox	0.631	0.944	33.2
Wolverine	0.588	1.00	41.2
Wolf	0.612	1.05	41.2
Black bear	0.583	1.21*	51.7
Polar bear	0.655	0.965	32.1

* *Average of two animals.*

which seemed to have much lower insulation in this study. It is possible that this difference represents a true climatic adaptation, the Alaskan hare having heavier winter fur than the Ontario hare.

Although seasonal differences were found in all species, by far the greatest absolute change was found in the larger animals i.e. those having the thickest fur. For winter pelts of animals there was roughly an increase of 0.25 insulation units for a 10-fold increase in body weight. Above about 1 kgm.

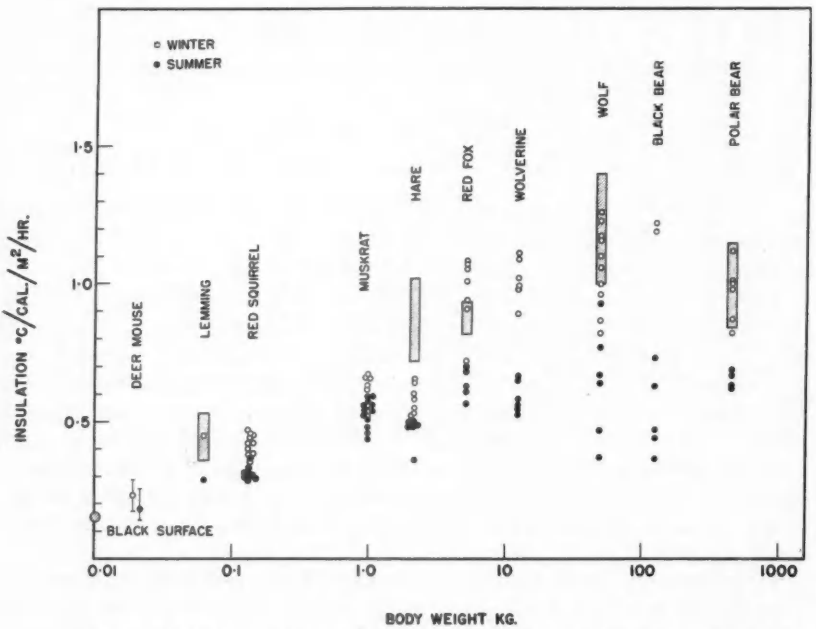

FIG. 2. Insulation of the fur in relation to body weight. Vertical cross hatched bars show data of Scholander *et al.* (12) for the same species.

for summer animals there was little or no increase in insulation. Evidently, very thick-furred animals such as the bears can reduce their insulation during the summer to a level about as low as that of the thin-furred muskrat. The common and marked seasonal change in insulation in the larger mammals may be regarded as an adaptation to high summer temperatures as well as to low winter temperatures, since heat dissipation in warm weather would be a major problem in animals with heavy fur.

Among the thin-furred mammals (2 kgm. and below), seasonal insulative change was obviously greatly reduced (Fig. 2). Thus small mammals, which have the greatest metabolic response to temperature changes (11), have only limited possibilities for seasonal insulative change. Therefore, unless these species entirely escape exposure to winter cold, seasonal acclimatization must largely lie in their ability to change heat production. On the other hand, even a limited seasonal change in the fur would enable the small animal to withstand lower temperatures in the winter than in the summer for the same energy expenditure. This effect might be further enhanced by lower skin surface temperatures in the winter. Thus, although small mammals in winter may be forced to expend extra heat for maintenance of body temperature, the cost would be reduced by these insulative adjustments. Observations on seasonal changes in metabolism (1, 2, 8, 10) give support to the notion that heat production in some species is actually less at a given low temperature in the winter than in the summer by virtue of greater insulation of the fur. The problem of variation in metabolic capability with season in small animals urgently requires further investigation.

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NITROGEN AND ARGON IN SALMONOID SWIMBLADDERS[1]

By J. S. Tait

Abstract

Analyses of 44 swimbladder gas samples from lake trout, lake whitefish, shallow-water cisco, deepwater cisco, and bloater caught at 60–300 ft. in Lake Huron showed no measurable quantities of combustible gas. Noncombustible gas ranged from 90 to 98%. The argon × 100/nitrogen in a gas sample from a whitefish was 1.02, virtually the same as in air. The noncombustible gas is composed apparently of the inert gases of the atmosphere.

Introduction

Saunders (2) in 1953 demonstrated that the swimbladders of the salmonoids contained extremely high pressures of gas that was neither oxygen nor carbon dioxide. He considered this gas to be nitrogen. The presence of such high pressures of nitrogen raised a problem of the mechanism for its deposition. However, there was still the possibility that the gas might be a combustible gas of organic origin. Scholander and van Dam (6) determined that in deep-sea marine physoclists organic gases play no significant role in the build-up of the gas pressure in the swimbladder. The investigation reported here was undertaken to determine whether the same is true for lake-dwelling salmonoids, which are primitive physostomes.

Materials and Methods

The fish whose swimbladders were sampled were caught in South Bay, Manitoulin Island, and immediately outside the bay in Lake Huron between July 24 and August 12, 1954. They were taken in gill nets at depths ranging from 60 to 300 ft. The gas samples were collected in hypodermic syringes immediately after the fish were brought to the surface by the method described by Saunders (2) and analyzed in a Fry Gas Analyzer which had been modified to burn combustible gas mixtures.

The modified Fry Gas Analyzer is shown in Fig. 1. The rubber mercury reservoir *g* was moved aside to allow a platinum coil to project into the glass bulb *e*. A mercury reservoir *j* and a levelling bulb *k* were added to allow for the expansion of gas during heating and combustion. When the screw clamp between *j* and *k* is closed the analyzer may be used in the usual manner (1).

Carbon dioxide was absorbed from the gas sample with 0.1 *N* potassium hydroxide and oxygen was absorbed with potassium pyrogallate (15 gm. of pyrogallol per 100 ml. of potassium hydroxide — sp. gr. 1.56). Then with the funnel filled with 0.5% sulphuric acid and with the upper meniscus of the sample two or three centimeters below the top of the pipette a measured volume of oxygen was introduced through the top by means of a syringe with

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Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ontario, with financial assistance from the National Research Council.

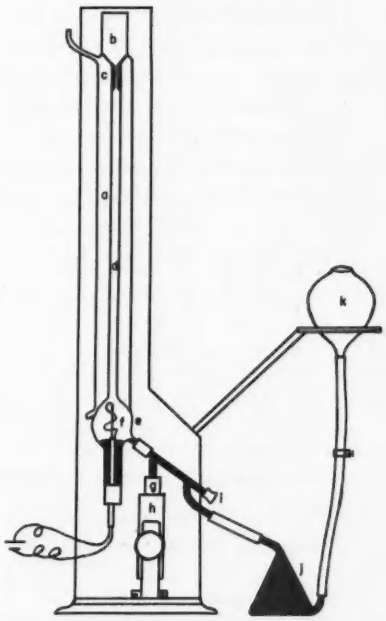

FIG. 1. Modified Fry Gas Analyzer. *a*, water jacket; *b*, funnel for introducing reagents; *c*, capillary of about 1 mm. bore; *d*, calibrated 1 ml. pipette; *e*, bulb of about 6 ml. capacity; *f*, platinum coil annealed in glass tube with leads to a 2 v. battery; *g*, rubber tube serving as mercury reservoir; *h*, metal tongue and adjusting screw for controlling mercury level; *i*, rubber vaccine cap; *j*, mercury reservoir; *k*, mercury levelling bulb.

a long needle which projected down through the capillary. As oxygen bubbled out of the needle it was drawn down into the bulb by withdrawal of the screw that presses on the rubber reservoir. The syringe was held in a device (4) adjusted to fill it to a volume of five milliliters.

The gas mixture filled the chamber so that the platinum coil was above the mercury level. With the funnel stoppered and the screw clamp open, the coil was heated to redness for one minute. The gas mixture was circulated past the hot coil by raising and lowering the levelling bulb. After ignition the clamp was closed, the stopper removed, and the excess oxygen and the carbon dioxide produced by combustion absorbed with potassium pyrogallate. The residual gas was measured to determine the loss in volume on combustion.

The oxygen used for dilution and combustion was stored over mercury in a glass flask equipped with a sidearm and vaccine cap. Five milliliter samples from the flask were tested in the analyzer to determine the amount of impurity, presumably nitrogen, being added in the five milliliters of diluent oxygen. The impurity found in commercial oxygen was always very close to 1%.

The efficiency of the combustion apparatus was tested by running checks on the combustion of propane–oxygen and propane–air–oxygen mixtures.

TABLE I

TEST OF COMBUSTION APPARATUS USING PROPANE AND PROPANE–AIR MIXTURES

Vol. propane, ml.	Vol. air	Before combustion V_1	After combustion V_2	Impurity in oxygen added N_2	Δ Vol. $V_2-V_1-N_2$
0.152	0	0.152	0.058	0.058	−0.152
0.157	0	0.157	0.056	0.058	−0.159
0.158	0	0.158	0.071	0.058	−0.145
0.143	0	0.143	0.054	0.058	−0.147
0.152	0.655	0.708	0.599	0.058	−0.167
0.170	0.614	0.664	0.573	0.058	−0.149
0.177	0.347	0.451	0.346	0.073	−0.178
0.154	0.326	0.411	0.310	0.060	−0.161
0.181	0.738	0.764	0.638	0.046	−0.172

TABLE II

ANALYSES OF SWIMBLADDER GAS FROM SEVEN DEEPWATER CISCO NETTED AT 300 FT.

Vol. sample, ml.	% Carbon dioxide	% Oxygen	Δ Vol. on combustion
0.817	1.2	2.0	− 0.006
0.812	0.6	6.7	+ 0.002
0.831	0.1	0.1	− 0.003
0.803	0.9	9.6	+ 0.004
0.693	0.3	5.2	+ 0.014
0.839	1.5	3.7	+ 0.019
0.838	1.2	7.9	− 0.007
			Mean = + 0.003

TABLE III

RESULTS OF ANALYSES OF SWIMBLADDER GAS FROM FIVE SPECIES OF SALMONOIDS

Species	No. of specimens	Depth range. ft.	Arithmetic means: % carbon dioxide	% oxygen	% Δ vol. on combustion
Lake trout, *Salvelinus namaycush* (Walbaum)	14	60–180	0.5	0.5	+ 1.6
Lake whitefish, *Coregonus clupeaformis* (Mitchill)	9	60–140	0.7	1.4	+ 2.3
Shallow-water cisco, *Leucichthys artedi* (Le Sueur)	7	60–180	1.3	13.5	+ 2.7
Bloater, *Leucichthys hoyi* (Gill)	7	140–300	0.6	5.1	+ 0.6
Deepwater cisco, *Leucichthys johannae* (Wagner)	7	300	0.8	5.0	+ 0.4

The results of some of the checks are shown in Table I. The loss in volume on combustion is practically equal to the volume of propane. The method was assumed to be sufficiently accurate to measure quantities of organic gas large enough to contribute to the build-up of pressure in swimbladders.

In addition a single 50 ml. sample from a whitefish was analyzed for argon. This analysis was kindly carried out by Dr. H. A. Shillibeer. The method used is described in Shillibeer and Russell (7). The sample was placed in a vacuum line where the argon was absorbed by activated charcoal in a liquid air cold trap and then purified by being heated in a calcium furnace, which removed all gases except noble gases. After purification the argon was measured in a McLeod gauge.

Results

Oxygen and Carbon Dioxide

Results of gas analyses for seven samples from deepwater cisco are shown in Table II. The summarized results for five species of salmonoids are shown in Table III. The percentages of oxygen and carbon dioxide agree with the values found for these species at corresponding depths in the much more extensive survey by Saunders (2).

Combustible Gas

The arithmetic means of change in volume on combustion (Table III) indicate a slight increase in volume. The small increase is attributed to experimental errors, and the change in volume is of course in the wrong direction to indicate combustible gas. Within the limits of accuracy of the method of analysis used there is virtually no combustible gas present in the swimbladders of these fish.

Argon

Three determinations of the argon content in the swimbladder of a whitefish taken at 100 ft. gave a figure corresponding to 0.99% of the total gas. Using the oxygen and carbon dioxide data for whitefish from Table III the argon $\times$ 100/nitrogen ratio was calculated. The resulting ratio of 1.02 differs little from the value of 1.18 for air. This is in accord with the findings of Schloesing and Richard (3) and Scholander (5) for deep-sea fishes. They found that the argon–nitrogen ratio in swimbladder gas was of the same order as that of sea water and the atmosphere. Scholander's values ranged from 0.87 to 1.61 in three species of deep-sea fish in which nitrogen content was less than 20%. It is most interesting to find that whitefish, which contain 97% nitrogen, also have an argon–nitrogen ratio the same as in air. The nitrogen in the swimbladder of this whitefish must have been deposited physically from gas in solution and not secreted chemically from an organic compound.

Acknowledgments

I wish to thank Dr. F. E. J. Fry who suggested and directed this investigation. The work was carried out at the South Bay Fisheries Research Station of the Ontario Department of Lands and Forests, and I am very grateful to the staff for their help. For argon analysis I am indebted to Dr. H. A. Shillibeer of the Department of Geophysics of the University of Toronto. Financial assistance was given by the National Research Council and made available through the Ontario Fisheries Research Laboratory.

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A STUDY OF THE LOCALIZATION AND DISTRIBUTION OF GLYCOGEN IN EARLY STAGES OF THE CHICK EMBRYO[1]

By David J. McCallion[2] and Wai Tuen Wong

Abstract

The localization and distribution of glycogen in the germ layers of chick embryos at stages 4, 5, and 6 (Lillie and Hamilton, 1952) was studied by means of the periodic acid – Schiff reagent as used by McManus (1948). In general the glycogen content of the embryos is greatest in the definitive streak stage, less in the head process stage, and least in the head fold stage. Glycogen is abundant in the ectoderm of embryos at stage 4, with a moderate decrease in stages 5 and 6. Glycogen is scarce or almost absent in the mesoderm and endoderm at all stages. There is a definite relationship between the amounts and distribution of glycogen and early developmental processes.

Introduction

It has been well known for a long time that glycogen along with other substances is the source of energy in cell division and other cell activities. Carbohydrate is the main source of potential energy in explanted chick blastoderms during the first four days of incubation (Spratt, 1948, 1950 (11, 12)). Glucose is essential for high cellular activity in the early chick embryo (Philips, 1941 (8)), and is essential to the continuance of the processes of morphogenesis and cellular differentiation (Spratt, 1948 (11)). Other sugars can replace glucose, but with less effectiveness.

In an extensive study of the early development of the avian embryo Jacobson (1938 (4)) included some studies on the distribution of glycogen in the chick embryo. It is surprising, in view of the extensive interest in the metabolism of the early chick embryo over the past 10 years (Spratt, 1952 (13)) that no attempt has been made to confirm this study and to correlate the information with recent studies on the metabolism of the embryo. It was for these reasons that the present investigation was undertaken.

Materials and Methods

The chick embryos used in this study were obtained from eggs of white leghorn hens. The eggs were incubated for a period of 18 to 25 hr. The incubated eggs were opened into a finger bowl containing physiological salt solution at about 39° C. The blastoderm was removed from the yolk under conditions that would preserve the glycogen content of the cells as nearly normal as possible. The embryos were staged according to Lillie and Hamilton (1952 (5)). The embryos were killed and fixed in chilled Rossman fluid (picro-alcohol–formalin), dehydrated in absolute alcohol and dioxan, embedded in paraffin, and sectioned serially. The sections were treated with

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Contribution from the Department of Biology, Acadia University, Wolfville, Nova Scotia.
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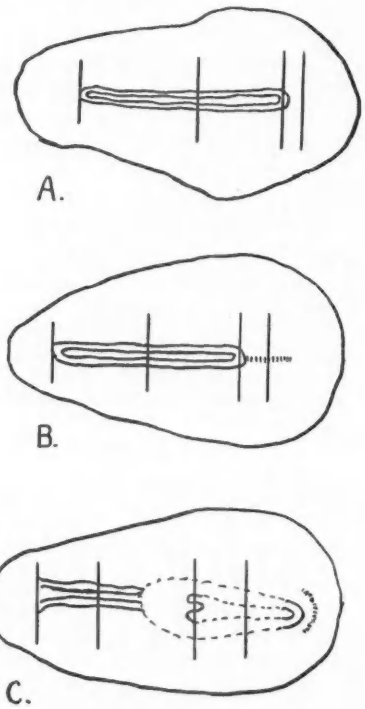

FIG. 1. Drawings of the stages of the chick embryo studied for the distribution of glycogen. The transverse lines indicate the levels at which particular observations were made. *A*. Stage 4. Definitive streak stage. *B*. Stage 5. Head process stage. *C*. Stage 6. Head fold stage. Drawn after photographs in Lillie and Hamilton.

the periodic acid – Schiff reagent as used by McManus (1948 (6)). After previous incubation of this material with saliva at 37° C. the results were negative.

This paper describes the results obtained from a study of six embryos at each of stages 4, 5, and 6 (Fig. 1) selected from a total of 40 embryos. Sections for study were selected at four levels in the embryo: anterior to the primitive streak, the primitive knot, the mid-region of the primitive streak, and the primitive plate region.

Observations

Anterior to the Primitive Streak

At this level, at stage 4, the embryo consists only of the ectoderm (epiblast) and endoderm (hypoblast), but the mesoderm is represented at stages 5 and 6. Glycogen is abundant in the ectoderm, showing only a slight decrease with age. It is more abundant in the outer layers of cells decreasing in the lower layers of cells. Glycogen is more abundant in non-nervous ectoderm than

in the medullary plate. The glycogen content of the endoderm was slight and nearly constant at the three stages studied. At stages 5 and 6 the notochord is present and also the mesoderm lateral to it. The glycogen content of the notochord and mesoderm in both stages is slight and much lower than in mesodermal cells at other levels.

The Primitive Knot Region

In this region, at all stages, there are the thin endoderm, the ectoderm, and the newly formed mesoblast cells spreading laterally between them. At this level the epiblast is thickened and modified to form the primitive knot. The cells of the ectoderm and the primitive knot are loaded with glycogen but with a gradation from the outer layers of cells to the inner layers. There is only a slight decrease with age from stage 4 through stage 6. Glycogen is scarce but present in the endodermal sheet, with a slight increase near the area opaca. The newly formed mesoblast cells contain very little glycogen in definitive streak stage embryos and even less in older stages. The glycogen content of the mesodermal cells is appreciably less than that of the endodermal cells.

The Mid-region of the Primitive Streak

At this level, in all three stages, there are three well-developed germ layers. Glycogen is abundant in the ectodermal cells and in the cells of the primitive groove. The amount of glycogen decreases somewhat from the outer layers of cells to the inner layers, and with age from stage 4 through stage 6. Glycogen is scarce but present in the cells of the endoderm. The mesoblast cells are poor in glycogen and the amount present decreases with the age of the embryo.

The Primitive Plate Region

The distribution of glycogen at this level is comparable to that in the middle of the primitive streak. The ectoderm is rich in glycogen while the mesoblast is poor in glycogen. Glycogen is present but slight in amount in the endodermal sheet.

Discussion

There are several methods for the visualization of glycogen, all of which have their virtues, but practical experience shows that the periodate–Schiff reagent as used by McManus (1948 (6)), following picro-alcohol–formalin fixation is to be preferred. This method reveals glycogen particularly well and loss of glycogen is minimal.

Glycogen has been demonstrated by a microchemical method in the endoderm of the amphibian gastrula (Heatley and Lindahl, 1937 (2)) but not by histochemical methods. Jacobson (1938 (4)) found no glycogen in the endoderm of the chick. Since the periodate–Schiff method depends upon the oxidation of adjacent hydroxyl groups in the glycogen molecule to aldehydes by periodate and subsequent recolorizing of the Schiff reagent, it probably

reveals both lyo-glycogen and desmo-glycogen (bound in a complex compound with other molecules). This would account for the glycogen that we have demonstrated in the endoderm.

Jacobson's (1938 (4)) principal study of glycogen was in the early stages of endoderm formation and mesoderm formation. We have studied the stages of mesoderm formation and distribution of organ-forming materials. At the stages studied there are extensive morphogenetic changes.

The spatial pattern of developmental activity characteristic of the early chick embryo is well-known (Spratt, 1952 (13)). The node at the anterior end of the primitive streak is the center of morphogenetic and histogenetic activity which results in the formation of the major axial structures as the node moves posteriorly. The forebrain is a secondary center of activity. That the pattern of developmental activity is underlain by a corresponding pattern of metabolic activity is demonstrated by the differential susceptibility of embryos to cyanide (Hyman, 1927 (3)) and differential staining with vital dyes (Child, 1925 (1), Rulon, 1935 (9), Miller, 1941 (7)). There is also evidence that regional differences in visible developmental activity are correlated with both qualitative and quantitative differences in metabolism, including respiratory metabolism (Spratt, 1952 (13)). Spratt (1952 (13)) has shown that morphogenetic processes (cell movements) as a group require less exogenous carbohydrate nutrient than processes of histogenesis. The distribution of glycogen in the cells of the germ layers and its disappearance from them during these morphogenetic movements seems to be correlated with all of these observations. The present study and that of Jacobson demonstrates very well the rapid utilization of glycogen during cell movements which is not recovered during the early phases of differentiation.

The primitive ridge and primitive groove clearly marks a boundary between glycogen-rich epiblast and glycogen-poor mesoderm. A similar disappearance of glycogen in the amphibian gastrula has been reported by Woerdeman (1933 (14)). In this respect, at least, the avian primitive streak is equivalent to the amphibian blastopore as a limit between glycogen-rich and glycogen-poor cells.

According to Spratt (1946 (10)) cell division and cell movement are the main factors in the invagination process. What factors are responsible for the loss of glycogen from the invaginating cells ? It was Jacobson's opinion that the developmental potencies and qualities of the endoderm and mesoderm are totally different from those of the epiblast from which they are derived and that the loss of glycogen is the first sign of this differentiation. Spratt (1950 (12)) has suggested that there is a fundamental energetic difference between form-building and tissue-building activities in the embryo. It could be assumed, therefore, that the loss of glycogen is a result of morphogenetic movements, particularly of epiblast cells into the mesoblast, since the mesoderm is poorer in glycogen than the older endoderm. This would also explain the moderate loss of glycogen in the epiblast cells migrating toward the blastopore from stage 4 through stage 6.

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INHIBITORS OF CARBONIC ANHYDRASE IN THE AMERICAN COCKROACH, PERIPLANETA AMERICANA (L.)[1, 2]

By Ann D. Anderson[3] and Ralph B. March

Abstract

Carbonic anhydrase activity has been demonstrated *in vitro* in preparations of the head, fat body, and gut of the American cockroach, *Periplaneta americana* (L.), and in the adult housefly, *Musca domestica* L. The insect factor, which is soluble in aqueous media and can be separated from the particulate cell fragments of insect tissue homogenates is heat labile and sensitive to cyanide inactivation. It is strongly inhibited by sulphanilamide, *p*-aminoethylphenylsulphonamide, and *p*-chlorphenylsulphonamide. No inhibition has been found with N-substituted sulphonamides or with any of the organic insecticides examined, including DDT, lindane, dieldrin, nicotine, rotenone, pyrethrins, and para-oxon. Sensitivity of carbonic anhydrase to sulphonamides having an intact—SO_2NH_2 group is also characteristic of mammalian preparations. The data indicate that inhibition of insect carbonic anhydrase cannot be an important factor in the mode of action of DDT or other organic insecticides.

Introduction

Studies of the biochemical mechanisms for the development of DDT (dichloro-diphenyl-trichloroethane) resistance in the housefly have demonstrated that resistant strains are able to detoxify DDT by dehydrochlorination to DDE (1,1-bis(*p*-chlorophenyl)2,2-dichloroethylene) to a greater extent and at a considerably more rapid rate than susceptible strains (14, 15, 16, 21, 22, 26). However, it is significant that intact DDT can be recovered from the bodies of living resistant flies more than 24 hr. after topical application in amounts which may be considerably greater than the LD_{50} for susceptible flies. This phenomenon may be taken to indicate that factors in addition to a degradation mechanism are involved in the biochemical adaptation of the housefly to organic insecticides. Such additional changes are also suggested by the development of resistance to prolan (1,1-bis(*p*-chlorphenyl)2-nitropropane) and to DDT plus synergists, which cannot be detoxified by dehydrochlorination (11, 12).

In an attempt to clarify this problem, we have undertaken the investigation of various enzyme systems in insects, with particular interest in determining their sensitivity to insecticides and other biologically active compounds. Reports by Torda and Wolff (24) and Keller (5) to the effect that low concentrations of DDT inhibit mammalian carbonic anhydrase preparations suggested that this enzyme, if present in insect tissues in measurable concentrations, should be examined as a possible site of attack by DDT.

Carbonic anhydrase, which catalyzes the reversible hydration of carbon dioxide to carbonic acid, is widely distributed in nature. It occurs in plants

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(13) as well as in animals. In animal forms in which the blood stream has an active role in respiration, carbonic anhydrase is essential in maintaining an adequate level of carbon dioxide exchange between blood and body tissues. It also functions in the adjustment of the acid–base equilibrium of inorganic ion-secreting organs (17, 23). Carbonic anhydrase has been identified in the haemolymph of only one insect, *Chironomus*; its larval form is unique among insects in having a respiratory pigment in the blood (19). Insect species in which the haemolymph has no active respiratory function lack this enzyme in the blood (2, 9). However, low levels of anhydrase activity have been found in the head, thorax, and abdomen of two species of Formicidae, in head and abdomen of *Musca musca*, and in abdomen and abdominal contents of *Popillia japonica* Newman (20).

Methods

A modification of the electrometric method of Wilbur and Anderson (25) was found to be satisfactory. This method, which consists basically of following the decrease in pH of a buffered solution as added CO_2 is converted to carbonic acid, was as follows: 2.8 ml. of $M/40$ or $M/20$ potassium phosphate buffer, pH 7.7, and 0.2 ml. of centrifuged tissue extract were mixed in a beaker, and the electrodes of a Beckman model H2 pH meter adjusted in the solution. The beaker was chilled continuously in an ice-water mixture to hold the temperature at 0°–2° C. Agitation was accomplished by a glass-enclosed magnetic stirring flea. The electrodes and all reagents were chilled. As rapidly as possible, 2.0 ml. of ice-cold CO_2-saturated distilled water were added to the buffered enzyme solution, and the decrease in pH was recorded at 10-sec. intervals. Since the uncatalyzed hydration of carbon dioxide proceeds at an appreciable rate, this rate and the standard deviation from the mean value obtained were calculated for both buffer concentrations. The catalytic influence of tissue preparations was considered to be significant when the mean rate was accelerated more than twice the standard deviation from the uncatalyzed rate. Raising the temperature from 0° to 15° C. during the determination approximately doubled the rate of hydration, which became too rapid for convenient measurement.

For comparison with insect extracts, a typical mammalian carbonic anhydrase extract was prepared from washed mouse erythrocytes by precipitating the haemoglobin from approximately four milliliters of blood obtained by heart puncture with 40% ethanol and chloroform. The extract was centrifuged and the sediment discarded. The resulting solution was stored in the refrigerator for four to five days without marked loss of activity. In contrast to the activity of mouse erythrocyte anhydrase extracted in this way, ethanol–chloroform preparations of cockroach and housefly tissues were completely inactive.

The terminal-instar American cockroach nymphs and adult female houseflies used in these experiments were reared in the laboratory on standard media. The flies were used three to four days after emergence from the puparium;

they were fed only 40% sucrose solution. Groups of 50 flies were homogenized with an all-glass Potter–Elvehjem homogenizer in 1.5 ml. distilled water or other solvent. Groups of 10 cockroach nymphs were dissected under CO_2 anaesthesia and the heads, fat bodies, emptied and washed guts with attached Malpighian tubules, and coxal muscles were pooled and homogenized in 2.0 ml. of water or 2% sodium fluoride in 5% detergent (Triton-X-100). The homogenates were allowed to stand at room temperature for 30 min., and were then centrifuged for 30 min. at 2780 r.p.m. The supernatant, which contained the carbonic anhydrase, was stored at 4° C. In the inhibition studies, the cockroach head homogenates were used exclusively: 2% sodium fluoride plus detergent was the extraction medium in every case, and the anhydrase determinations were made in $M/20$ buffer.

Inhibitors were added to the buffer–enzyme mixtures at room temperature. The sulphonamide derivatives and other water–soluble compounds were added in 0.2 to 0.4 ml. of distilled water. Those insecticides which were not appreciably water soluble were introduced in acetone or alcohol to form fine suspensions. Detailed studies of the range of inhibitory concentrations of the active compounds were not undertaken. Each substance investigated was first tested at a concentration of approximately 4×10^{-3} M in the total volume, then retested at 1×10^{-2} M if found to be inactive at the lower level. Compounds which did inhibit were re-evaluated at lower concentrations. Inhibitory effect was first determined immediately after the inhibitor was added to the buffered enzyme solution. Compounds which failed to inhibit under these conditions were then incubated with the enzyme extract for 30 min. at room temperature before measuring carbonic anhydrase activity. The materials which proved to be without effect if added just before anhydrase determination also failed to inhibit after incubation with the extract. Strong inhibition was obtained with cyanide and the sulpha compounds without prior incubation, and all of the data obtained for these inhibitors represent the shorter procedure.

Results

The soluble factor which catalyzes the reversible hydration of carbon dioxide to carbonic acid *in vitro* was found to be widely distributed in cockroach tissues. From Table I it can be seen that the catalytic potency of the extracts is influenced to a certain extent by the extraction medium. Sodium fluoride, which does not interfere with carbonic anhydrase measurement, was the preferred solvent because of its bacteriostatic property. Detergents have been shown to increase the activity of soluble enzymes in homogenates which contain a high percentage of fat (23). Fat body is much richer in lipids than other insect tissues, and a pronounced increase in the anhydrase activity of extracts of this tissue in the presence of a surface-active agent would be expected. Using the more active fluoride extracts it was possible to double the concentration of buffer used, and thus to increase the difference in rate of hydration between the blank and the catalyzed reactions without affecting the

TABLE I

QUALITATIVE DETERMINATION OF AMERICAN COCKROACH AND HOUSEFLY CARBONIC ANHYDRASE: EFFECT OF VARYING CONDITIONS OF EXTRACTION AND MEASUREMENT. VALUE FOR MOUSE ERYTHROCYTE ANHYDRASE REPORTED TO SHOW ORDER OF ACTIVITY OF MAMMALIAN PREPARATION

Extraction medium	Determination medium	Tissue extracted	Time in sec. pH to drop 7.7 to 6.85 after CO_2 addition
H_2O	*M*/40 phosphate buffer	None	72 ± 14 (32 det.)
Ethanol–chloroform	*M*/40 buffer	Erythrocytes, 4 ml. mouse	8*
H_2O	*M*/40 buffer	50 houseflies	28
H_2O	*M*/40 buffer	10 cockroaches:	
		fat body	31
		coxal muscle	44
		head	20
		gut	25
2% NaF + detergent	*M*/20 buffer	None	114 ± 26 (48 det.)
2% NaF + detergent	*M*/20 buffer	10 cockroaches:	
		gut	28
		fat body	14 ± 6 (13 det.)
		head	15 ± 6 (34 det.)

* *Average of 3–10 determinations unless number of replicates stated.*

activity of the latter. It should be pointed out that after the addition of CO_2-saturated water to the buffered system, the pH fell rapidly in the presence of tissue extracts to a lower value usually in the range 6.0 to 6.3, and remained constant at that value. Differences in the terminal pH occurred, and were taken to be an indication of the concentration of enzyme present: in the presence of a greater or lesser amount of carbonic anhydrase more or less carbonic acid would be formed before the equilibrium $CO_2 + H_2O \rightleftharpoons H_2CO_3$ is re-established. Blank determinations lacking enzyme only underwent a gradual decrease in pH. The terminal value was pH 6.8 to 6.9, and indicates that a much smaller concentration of carbonic acid was formed spontaneously than in the presence of a catalyst. A significant fraction of the added carbon dioxide undoubtedly escaped into the atmosphere during the course of the determinations, resulting in a decreased substrate concentration with time.

Since carbonic acid formation is catalyzed by a variety of inorganic substances (6, 23) the sensitivity of the insect extracts to treatments which denature enzymes in general or vertebrate carbonic anhydrase in particular was surveyed. The inhibition study is summarized in Table II. Heating the insect extract in buffer to 80° C. for 10 min. reduced or abolished the catalytic effect, as did low concentrations of cyanide. Potassium permanganate was only slightly inhibitory at high concentrations, in contrast to its potency against purified mammalian anhydrase (7). This difference might

TABLE II

INHIBITORS OF CARBONIC ANHYDRASE ACTIVITY IN UNIFORMLY PREPARED EXTRACTS OF AMERICAN COCKROACH HEADS

Inhibitor	Concentration	Time in sec. pH 7.7 to 6.9
None	—	15 ± 6*
Heat: 80° C.	—	82
NaCN	.0005 *M*	120
$KMnO_4$	> .0075 *M*	25
Sulphanilamide	.0015 *M*	40
	.0029 *M*	48
	.0058–.01 *M*	105–50
	.05 *M* and up	116
p-Aminoethylphenylsulphonamide	.0005 to .0066 *M*	72
p-Chlorphenylsulphonamide	1/10–1/100 saturated	130
Sulphamerizine	See text	18
Sulphathiazole	"	14
Sulphasuxidine	"	12
Morpholine	"	16
Nicotine	"	23
DDT	"	20
Lindane	"	21
Dieldrin	"	18

* *Standard deviation: see Table I.*

be due to the protective action of impurities in the crude insect extracts or to an intrinsic variation in sensitivity. Reducing the volume of a given enzyme preparation caused a corresponding and proportional reduction in the rate and extent of hydration of carbon dioxide. These observations favor the conclusion that the insect factor is an enzyme. Sulphonamides having an intact—SO_2NH_2 group were strong inhibitors of cockroach preparations.

From Table II it can be seen that the chlorinated hydrocarbon insecticides, and indeed all of the insecticides examined, were without effect on this factor. Other insecticides which were tested and found to be innocuous were para-oxon, rotenone, pyrethrins, isobornyl thiocyanoacetate, and 4,6-dinitro-*o*-cresol. Carbonic anhydrase extracts prepared from the heads of cockroaches injected 24 to 48 hr. previously with toxic doses of DDT, lindane, nicotine, or dieldrin underwent no impairment of activity. However, similar experiments with nontoxic doses of sulphanilamide were equally negative, which leaves the area of *in vivo* interactions open to question. Because no specific effect of insecticides on cockroach carbonic anhydrase was found, it was not considered necessary or profitable to extend the investigation to the housefly.

Discussion

The functions of carbonic anhydrase in intact insects remain to be explored. Insect blood functions passively in transporting carbon dioxide (19). From 30 to 50% of the carbon dioxide in the haemolymph is carried as dissolved gas; the rest is bicarbonate (8). The absence of measurable levels of anhydrase activity from haemolymph is probably related to the absence of an active

respiratory function. This conclusion is supported by the statement of Robertson and Ferguson (18) that carbonic anhydrase has never been found in fluids free of intact or lysed cells, and appears to be a cellular constituent only. Because molecular carbon dioxide migrates much more rapidly than the carbonate ion across cell membranes, carbonic anhydrase may be essential in insect respiration in accelerating the excretion of catabolic carbon dioxide into the tracheoles (3). The important possibilities that this enzyme is essential to the excretion of inorganic ions or that it plays a part in biological syntheses involving the fixation of respiratory carbon dioxide also deserve investigation.

The sensitivity of the cockroach extracts to unsubstituted sulphonamides and their immunity to inhibition by N-substituted sulphonamides parallel the reaction of mammalian carbonic anhydrase. Mann and Keilin (10) first reported that the sulphonamide group is directly responsible for the inhibition of the mammalian enzyme, and suggested that it may react directly with the zinc-containing moiety of the molecule. A similar molecular structure might be postulated for insect carbonic anhydrase on the basis of this observation.

In contrast to the earlier observations of Torda and Wolff (24) and Keller (5) with mammalian carbonic anhydrase, high concentrations of DDT failed to give detectable inhibition of cockroach anhydrase under the conditions of the experiment. The degree of dispersal of the insecticides obtained by adding acetone or alcohol solutions to an aqueous reaction medium has been found to be quite sufficient to give good inhibition of sensitive enzymes (4), and we feel that the failure of DDT to inhibit cannot be explained on this basis. Longer incubation of the enzyme with the insecticide prior to determination, or the use of a more sensitive method for measuring the activity might disclose an inhibitory effect, but such an effect must be very low in comparison with that of sulphanilamide. It appears very unlikely that carbonic anhydrase is involved in the toxic action of DDT.

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NOTES

PHYSALOPTERA VARIEGATA REIBER, BYRD AND PARKER, 1940 FROM THREE SPECIES OF REPTILES

BY PATRICIA M. MAWSON

Through the courtesy of Dr. W. Hamilton, Jr., of Cornell University, specimens of *Physaloptera* were received from *Coluber constrictor constrictor*, *Ophiosaurus ventriculus*, and *Heterodon platyrhinos*. All were collected at Fort Benning, Ga., U.S.A. The specimens from *C. c. constrictor* and *O. ventriculus* were identified as *P. variegata* Reiber, Byrd and Parker, 1940, and constitute a new host record for the latter. Only females were recovered from *H. platyrhinos* and, because it is impossible to identify a species of *Physaloptera* from females alone, they are only tentatively referred to *P. variegata* even although they appear to be identical with those from the other two snakes.

Morgan (2), in reviewing the species of *Physaloptera* of reptiles, re-examined the type specimen of *P. abjecta* (1, 4) and, although it was an immature female, he regarded *P. variegata* Reiber, Byrd and Parker, 1940 (3) as a synonym.

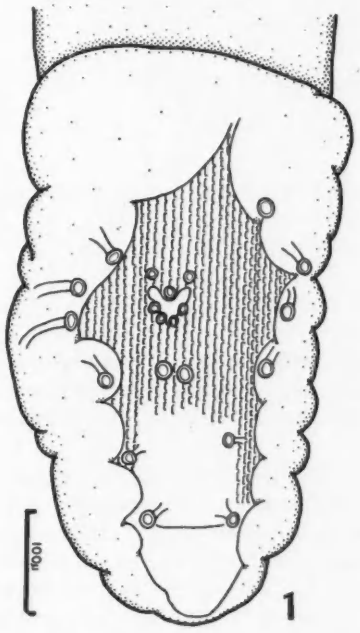

FIG. 1. Ventral view of male tail.

Although he recorded the species from *Thamnophis s. sirtalis* and *Liopettis vernalis*, he did not describe his specimens and it must be assumed that they agreed with those of Reiber, Byrd and Parker. Because *P. abjecta* is an immature female and because it was recorded from *Psammophis flagelliformis*, *P. variegata* cannot be regarded as a synonym of *P. abjecta*, which should be considered as a species inquirenda.

The present specimens differ from the description of *P. variegata* in having a much longer vagina (1.8 mm.) which divides into two uteri near the base of the oesophagus. In the males, the spicules are longer (570 to 620μ and 320 to 360μ). There are three pairs of preanal papillae and seven of postanal (Fig. 1), the first four of which are close to the anus while the posterior three are evenly spaced on the tail.

These differences are not considered sufficient to remove the present specimens from *P. variegata*.

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A POSSIBLE TEST FOR NEMATODE VIABILITY

BY KEITH RONALD

In the course of routine "black-light" candling for parasites in the flesh of the Atlantic cod (*Gadus callarias* L.), it was observed that in certain samples of fillets the worms were not fluorescent. Fluorescence, when present, enables one to obtain a more accurate estimation of the incidence of metazoan parasites in the flesh than can be obtained by the use of the usual transmitted-light method.

Upon investigation it was found that the only apparent difference was that some samples of fish were frozen whi e others were not. The frozen samples showed the codworm under "black-light" illumination whereas the fresh samples did not. Some of the non-fluorescent worms were removed from fresh fillets and placed in boiling water; they were then replaced in the fillet and candled by the "black-light" method. They were then seen clearly, indicating that only the dead worm is fluorescent. As a further experiment,

living worms, chosen by their active movements, were placed in a solution of one part sea-water to two parts distilled water and held at room temperature. These were examined daily for signs of movement and fluorescence. It was observed that movement ceased approximately 30 hr. before fluorescence occurred, suggesting that immobility in helminths is not always a sign of mortality. When these observations were being made, the source of "black-light" was never used for a longer period than 10 min., in an attempt to minimize the effects of the ultraviolet emission. Fluorescence persists for an extended period of time and has been observed up to 30 days after its first appearance.

It is not clear why dead worms should be fluorescent. Coagulation of the protein may be involved rather than bacterial action because the application of boiling water brings about instant fluorescence. This procedure, with slight modification, suggests possibilities for various tests of helminth viability.

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